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PHYTOPATHOLOGY

VOLUME XII NUMBER 10

OCTOBER, 1922

SEPTORIA DISEASES OF CEREALS.[1]

GEORGE F. WEBER

WITH PLATES XXIX AND XXX AND FIVE FIGURES IN THE TEXT

INTRODUCTION

It is the purpose of the present paper to bring together the results of investigations conducted during the past two years at the University of Wisconsin on the diseases of cereals and related plants caused by certain species of Septoria. The investigations have involved a study of these diseases in the field, greenhouse and laboratory as well as a study of herbarium material and a review of the literature. It has been found that this group of diseases is caused by closely related species of Septoria. However, by means of morphological and cultural studies of the organisms and inoculation experiments it has been possible to segregate these diseases very clearly.

The disease on oats has been found for the first time in the United States, and the ascigerous stage of the causal fungus has been developed in artificial cultures. This ascigerous stage has not as far as known been found under natural conditions. This fungus on oats has been found to be sharply specialized to the genus Avena. The common name "Speckled Blotch" of oats is here suggested for this disease on account of the speckled condition of the lesions caused by the presence of the scattered, black pycnidia. Accordingly the results of these investigations are given under the heading "The Speckled Blotch of Oats," caused by Leptosphaeria.

The investigations have verified previous work in showing that there are two diseases of wheat caused by two rather closely related species of Septoria. The results on these two diseases of wheat are given under the headings of "The Glume Blotch of Wheat" and "The Speckled Blotch of Wheat," respectively.

It has been found that the disease on rye, barley, quack grass (*Agropy-*

[1] This is the first of three articles by the author on Septoria diseases of cereals and some of the grasses.

ron repens), brome grass (*Bromus inermis*) and Kentucky blue grass (*Poa pratensis*) are each caused by distinct species of Septoria. In each case the fungus seems to be specialized to the genus or host on which it occurs. The results of these investigations are given in the order in which the hosts are mentioned above.

The seeds necessary for the propagation of the plants were obtained from the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

The different species of Septoria were collected in the field in the vicinity of Madison, Wisconsin. During the past two years, observations have been made in the cereal plots on the Hill Farm of the University of Wisconsin, and in the plots of the Wisconsin Experiment Station at Madison, Wisconsin.

The inoculum used in the various experiments was obtained from pure culture and from diseased material collected in the field. The plants were inoculated by means of an atomizer, usually by spraying a spore suspension on the leaves of the host plants. In the field the inoculated plants were then covered with glassine bags for from 48 to 72 hours depending somewhat on weather conditions. The bags were then removed and the data were taken after about two weeks. In the greenhouse the inoculated plants were placed in a moist chamber for from 2 to 4 days and then placed on a greenhouse bench.

The writer takes pleasure in making acknowledgment to Dr. A. G. Johnson, for helpful suggestions, criticisms and guidance during the progress of the work, for aid in preparing this paper, and also for access to his collections of Septoria diseases on various cereal hosts collected at different points in the United States; also to Dr. J. J. Davis for herbarium material, literature references, and many helpful suggestions.

The writer also wishes to express his appreciation for the assistance rendered by Dr. Etienne Foex of Paris, in kindly lending at the request of Dr. A. G. Johnson, portions of type material from the original collections of J. B. H. J. Desmazieres.

I. SPECKLED BLOTCH OF OATS CAUSED BY LEPTOSPHAERIA

INTRODUCTION

A disease of oats caused by *Septoria avenae* Frank, previously referred to by the writer (9), was found near Madison, Wisconsin, in September, 1921. The disease occurred on volunteer oats on the University farm. The plants were heavily attacked by crown rust and the scattered lesions

caused by Septoria, one to four on a leaf, occurred promiscuously intermixed with the rust sori (Fig. 1). The Septoria lesions were evident as rather small, circular to elongate, elliptical, killed and faded areas 2 to 4 by 2 to 8 mm. in size. The lesions were definitely distinguished from other spots on the leaf only by the presence of black, more or less scattered pycnidia. The development of the lesions as observed in inoculation experiments, discussed later, is briefly as follows:

The first visible symptoms after inoculation were observed on the eight or ninth day. Then spots in the leaf were readily detected. These spots were slightly lighter in color than the healthy parts. After 12 days the leaves were very mottled and the pycnidia could be distinguished. They were a light brownish color and very small. After 14 days the pycnidia turned black and most of the yellowed areas died forming spots light yellow to dirty white in the center, surrounded by a band of dull brown, and the whole surrounded by yellowish tissue blending into

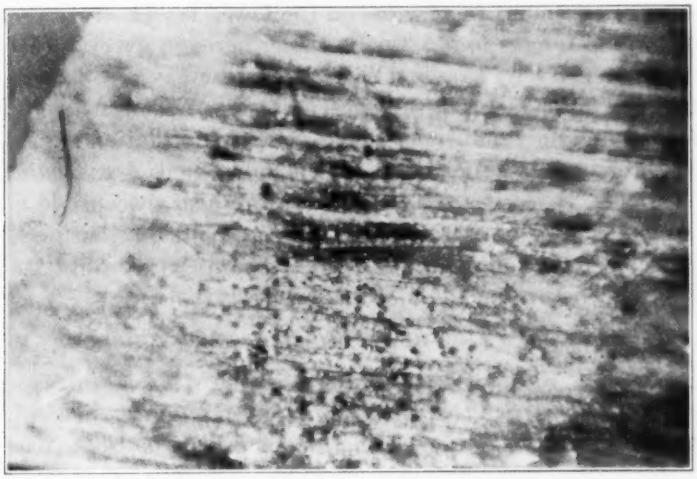

Fig. 1. Portion of an oat leaf collected near Madison, Wisconsin, showing lesion caused by *Leptosphaeria avenaria*. Note black pycnidia in the lesion. The leaves also show a heavy attack of crown rust, *Puccinia coronata*. September, 1921. × 26.

green. The central portion of the spot was dotted with scattered pycnidia. Infected oat plants were found only in two fields about half a mile apart; while other oat fields in the vicinity were carefully inspected, no evidence of the disease was found in them.

So far as the writer has been able to determine, the disease has not been previously reported in the United States. It has, however, been

found several times previously in other parts of the world. In 1847 Desmazieres (4) reported from France a Septoria disease on oats, and after examining it classified it as variety *C. avenae* of *Septoria graminum* Desm. In 1866 Moriere and Roberge (7) found also in France a Septoria disease on cultivated oats and listed it as *Septoria graminum* var. *C. avenae* Desm. Through the kindness of Dr. Etienne Foëx, Director of the Station de Pathologie Vegetale, Paris, portions of the type material of *Septoria graminum* var. *C. avenae* have been examined and found to be distinct from *Septoria avenae* Frank. The pycnospores as described are identical with *Septoria graminum* Desm. Hence the pycnospores of var. *C. avenae* Desm. are longer and distinctly narrower than *Septoria avenae* Frank. In 1892 Cobb (3) reported a Septoria disease on oats occurring in Prymble, England. He gave no description of the disease or organism nor did he mention the species of oats attacked. In 1895 this disease was reported on oats in Pomerania, Germany by Frank (6). He gave a complete description of the organism and named it *Septoria avenae* n. sp. He found the disease on the lower leaves of *Avena sativa*. The disease and fungus found by the writer agree in every respect with the description given by Frank. Thus the disease has now been reported from England, Germany, and the United States (Wisconsin). In nature it has been found only on *Avena sativa*. From inoculation experiments, reported later in this paper, it has been found that *Avena barbata*, *A. brevis*, *A. nuda*, *A. strigosa*, and *A. fatua* are also susceptible. Hence in the United States, up to the present time, this disease has been found developing naturally only to a limited extent on *Avena sativa*, and thus far it seems to be of negligible economic importance.

TAXONOMY AND MORPHOLOGY OF THE FUNGUS

In 1895 Frank (6) described a *Septoria sp.* on *Avena sativa* from Germany and named it *Septoria avenae* n. sp. The writer in September, 1921, found a *Septoria sp.* on *Avena sativa* near Madison, Wisconsin, which agreed with the description of Frank. The fungus was isolated by making single spore isolations and grown on potato-dextrose-agar and oat-meal agar. A number of transfers were made to each of these kinds of agar. In January, 1922, perithecia, with mature asci, were found both in the oatmeal agar cultures and in the potato agar cultures. In each case, as previously stated, cultures were made by isolating single pycnospores. They were obtained from pycnidia from oat leaves collected in the field. To date (April, 1922), perithecia have been found in at least fifty different culture tubes. Mature pycnidia were also found in practically all of the cultures. The ascospores germinated in

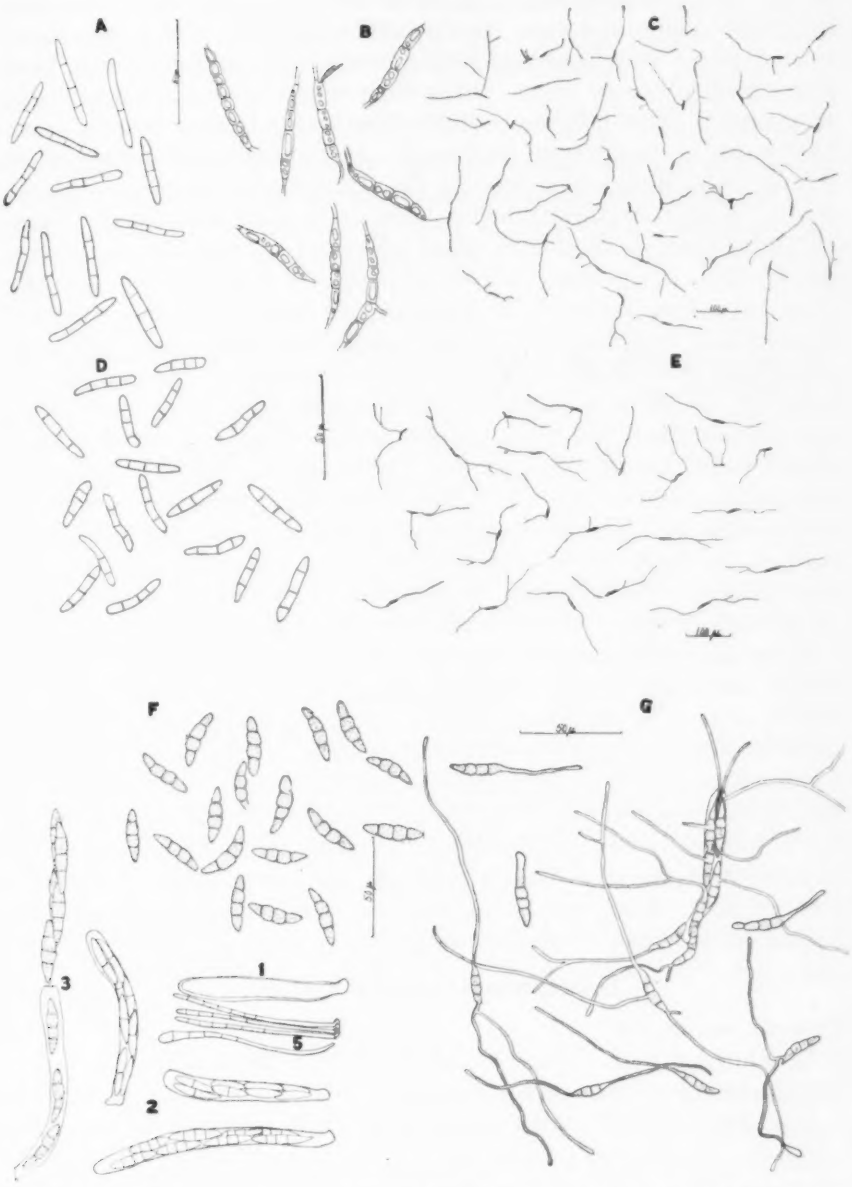

Fig. 2, A. Pycnospores developed on oat leaves in the field. B. Vacuolate condition of pycnospores 36 hours after germination. C. Germination of pycnospores after 24 hours, collected on oat leaves. D. Pycnospores grown on potato-dextrose agar. E. Germination of pycnospores after 24 hours, grown on potato-dextrose agar. F. Asci: (1) empty, (2) immature, (3) mature, (4) ascospores and (5) paraphyses of *Leptosphaeria avenaria*. G. Ascospores germinating in water after 18 hours. (Camera lucida drawings).

water in about four hours and on potato agar in seven hours. Ascospores in a water suspension were floated out on the surface of potato agar, poured plates and incubated twelve hours. Single spores were then transferred to culture tubes. After three weeks typical pycnidia began to appear in these cultures. About three weeks later perithecia began to develop and soon were numerous. Using ascospores developed in culture, inoculations were made in the greenhouse on seedlings of *Avena sativa*. Typical Septoria infections resulted in about two weeks. These infections were identical with other infections produced by inoculating oat seedlings of the same age with pycnospores obtained from pycnidia from oat leaves collected in the field. In both series the reisolated pycnospores from the inoculations made with ascospores and those made with pycnospores could not be distinguished. Pycnospores collected in nature were cultured and compared to cultures made from pycnospores developed from ascospores and those made with pycnospores could not be distinguished. Pycnospores collected in nature were cultured and compared to cultures made from pycnospores, developed from ascospores. The two strains of pycnospores, germinated in the same manner, the hyphae were septate, of the same size and branched in the same manner. The mycelium in general was of the same color and growth in the culture was of the same sort in each case.

From this evidence it is clear that the ascigerous stage of *Septoria avenae* has been found. From its characteristics, described later, it clearly belongs in the genus Leptosphaeria. It seems to be distinctly different from any described species of this genus. The fungus described by Auerswald (1) in 1869, on oats as *Leptosphaeria avenae* is distinct in that the ascospores of that fungus are considerably smaller, measuring 3.5 to 4 by 15 to 16 μ and are hyaline, in contrast to the larger, colored ascospores of the form found to be the ascigerous stage of *Septoria avenae* Frank. Therefore the following new combination is proposed and a description of the stages follows:

Leptosphaeria avenaria sp. nov.

Septoria avenae Frank
Berichte der Deut. Botan. Gesell. **13**: 61–65. 1895.
Not *Septoria graminum* var. *C. avenae*
Ann. Des. Sci. Nat. 3rd Serie **8**: 9–18. 1847.

PYCNIDIA

Pycnidia are more or less scattered, often in rows, sub-epidermal, globose to sub-globose, visible to the unaided eye, 90–150 μ in diameter,

averaging about 120 μ. Wall is smooth, brown to black in color, composed of one to three layers of pseudoparenchymatous cells. Ostiole, round to oval, slightly elevated, 20–30 μ in diameter.

PYCNOSPORES

Pycnospores (Fig. 2, A and D) are rod shaped, straight or slightly curved, cylindrical, with rounded ends, three septate when mature, hyaline, guttulate, usually one or more guttulae on each side of the septa, 3–4 × 25–45 μ averaging 3.5 × 38 μ.

PERITHECIA

Perithecia were grown in test tubes at room temperature from pycnospores on potato-dextrose-agar, slightly acid. They were slightly embedded, globose to sub-globose, 60–130 μ in diameter. Walls smooth, black pseudoparenchymatous, thin, 2–3 cells thick. Ostiole usually round, not protruding, 12–20 μ in diameter.

ASCI

Asci (Fig. 2, F) are narrowly clavate with rounded tips, hyaline, thin walled, 10–18 × 30–100 μ, averaging 15–50 μ. Each ascus contained eight spores biseriately arranged.

ASCOSPORES

Ascospores (Pl. XXIX B and C) are fusoid, straight or slightly curved, ends obtuse, rounded, three septate, constricted at the septa, especially the center septum, second cell from top usually swollen, light yellow to slightly olivaceous, 4.5–6 × 23–28 μ, averaging 5 × 25 μ.

PARAPHYSES

Paraphyses (Fig. 2, F) are narrowly cylindrical, hyaline, septate, with slightly enlarged, rounded tips, 2 × 60 μ, terminal cell 2–4 μ in diameter.

MYCELIUM

Mycelium grown in culture from either pycnospores or ascospores is hyaline, much branched, septate, with hyphae about 2 μ in diameter. The contents of the younger hyphae are either homogeneous or very minutely guttulate especially near the septa. The older hyphae become distinctly vacuolate. The mycelial colonies grown on agar are white with a very slight pinkish tinge.

PHYSIOLOGICAL STUDIES

Cultural Studies

The fungus grows readily in artificial culture. Numerous media were used and the mycelium developed well on all of them except string-bean agar in which case the surface seldom was completely covered and the growth was usually scant. Characteristic growth was produced on sterilized corn meal, wheat heads and stem of *Melolitus alba*. The most favorable media proved to be potato-dextrose agar and oatmeal agar. The growth on these two media was especially vigorous and rapid. Pycnidia began to form at the end of about four weeks and at the end of about eight weeks mature perithecia were found on both of these media. The pycnospores and ascospores developed in the pycnidia and perithecia respectively, germinated readily. The mycelium developed by the pycnospores grew on potato-dextrose agar very much the same as the above description. The mycelium from the ascospores on potato-dextrose agar developed rapidly and was of a slightly pinkish white color when several days old. After about two weeks pycnidia began to form along the upper edge of the slant where the medium was thin (Pl. XXIX, A). The formation of the pycnidia was accompanied by a change of color of the mycelium from white to grayish. After four weeks perithecia were also found in the same tubes in which the pycnidia were found. Both forms were mature and compared exactly with the pycnospores germinated after 3–4 hours in water in the manner described later.

Germination Studies

Pycnospores. Pycnospores (Fig. 2, B, C and E) taken from the pycnidia on oat leaves collected in the field as well as those grown in pure culture on culture media showed indications of developing germination tubes after three hours at room temperature, when placed in a drop of water on a glass slide. When placed on the surface of potato-dextrose agar no evidence of germination was observed until after eight hours. The pycnospores usually produced germ tubes from each end and occasionally from the sides. Four germ tubes have been observed developing from a single pycnospore in water. The most favorable temperature for spore germination was between 20–24° C. At this temperature the spores germinated in the shortest time and the growth was most rapid and vigorous. At the lower temperatures the time before germination was lengthened, although germination took place at 4° C. after 48 hours. At the higher temperatures the time for germination was shorter but the

germ tubes did not appear to be as turgid. No germination took place above 36° C.

Ascospores. Ascospores (Fig.2, G) developed on potato-dextrose agar and oatmeal agar were removed from the perithecia and placed in distilled water on glass slides or placed on clarified potato agar. The spores in distilled water showed the formation of germ tubes after four hours at room temperature. On the potato media the first germ tubes were not observed until after seven hours. The germ tubes were developed first at the ends of the spore and soon after one of the remaining two

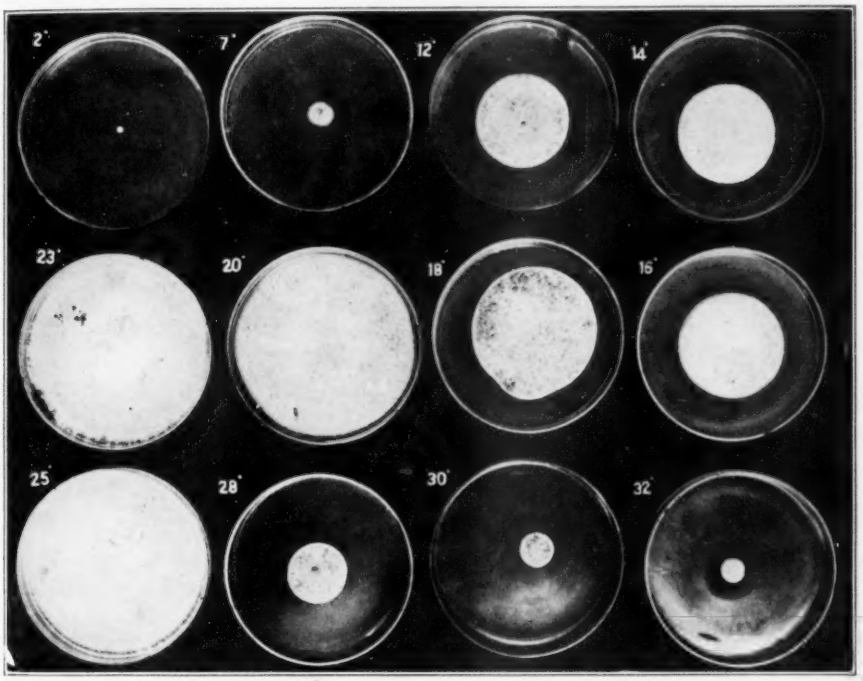

Fig. 3. *Leptosphaeria avenaria* grown 12 days on potato-dextrose-agar at various centigrade temperatures as indicated, showing extent and type of growth of each. One fourth natural size.

cells developed a germ tube. The germ tubes were hyaline, rounded at the growing tip, septate, and immediately sent out branches forming a mycelial mat. Ascospores retained within the ascus germinated (Fig. 2, G) as soon as those that were free in the water. The germ tubes from these ascospores broke through the ascus wall at many different places

along the sides as well as growing from the openings at the tip and base of the ascus. It was also observed that ascospores which were accidentally broken into two parts germinated from the end cells in the same time as uninjured spores. When germinating in a drop of water on a glass slide the ascospores and germination tubes adhered very persistently to the glass surface, remaining in position in running water.

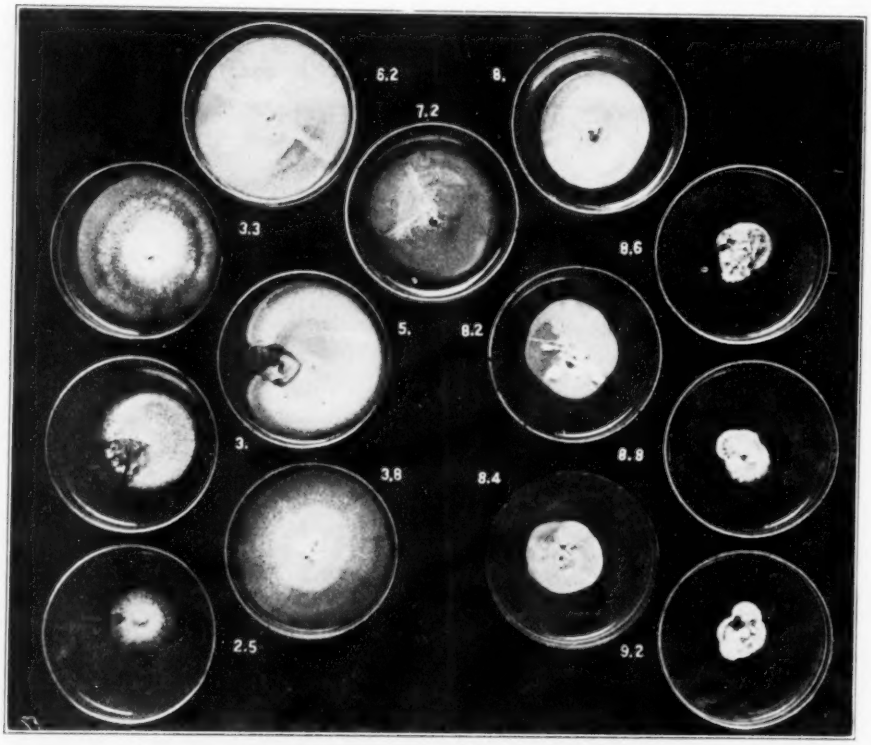

Fig. 4. Organism grown on acid and alkaline potato-dextrose-agar adjusted to the pH values as indicated, showing extent and type of growth to each reaction. One-fifth natural size.

The superfluous water was removed from a group of ascospores which had germinated twelve hours previously and diluted India ink as used by Errera (5) and Boyle (2) was placed on the ascospores and then covered with a cover slip. Examination with the microscope showed that a light colored area surrounded every germinated ascospore and also the older portions of the germination tubes. At the growing tip

there was little or no evidence of this gelatinous sheath. It gradually increased in extent toward the spore (Pl. XXIX, B). No doubt, as pointed out by Boyle (2) gelatinous sheaths of this sort may play important rôles in initial infections in making it possible for the spores and germination tubes to adhere tightly to the cuticle of the host.

The Relation of Temperature to the Growth of the Organism in Culture

The relation of temperature to the growth of the fungus was studied by growing the mycelium on poured plates of potato-dextrose agar incubated at different temperatures (Fig. 3). Bits of mycelium were transferred to the center of each plate from a common source and the plates were placed in incubators and left there until the mycelium at the most favorable temperature had covered the surface of the plate. It was found that little or no growth took place at 2° C.; the most abundant growth was at 20–25° C. From 25° C. upward growth decreased until at 32° C. where very little growth took place. Hence the cardinal temperatures for mycelial growth would be approximately as follows: minimum 2° C., optimum 23° C., maximum 32° C. There were decided decreases in growth below 12° C. and above 25° C.

The Relation of the Reaction of the Medium to the Growth of the Organism in Culture.

The fungus was grown on potato-dextrose agar to which had been added different amounts of $\frac{n}{1}$ HCl or $\frac{n}{1}$ NaOH. The agar was prepared and made neutral to phenolphthalein, then 10 cc. of the medium were placed in each test tube to which was added one or more drops of $\frac{n}{1}$ HCl or $\frac{n}{1}$ NaOH. Thirteen tubes made up a series which was found to range from pH 2.5 to pH 9.2 (Tab. I). Ten series were made up in the same way. Five series were used as controls, two were tested for acidity by the Fuller scale and the other three by Clark and Löeb's colormetric method for determining H-ion concentrations. The five remaining series were poured into petri dishes and inoculated with the fungus from a single source, and placed at room temperature in the dark for growth. The experiment was concluded when the fungus covered the surface of any petri dish. After fourteen days the pH 6.2 petri dish cultures were covered by the fungus. The growth was decidedly less at pH 7.2 and gradually decreased up to pH 9.2 where very little growth had taken place. From pH 8.6 to pH 9. 2 the growth of the fungus was not characteristically normal. The margins were irregular, some dark spots appeared and the surface of the colony was very irregular when

compared with the colonies in other petri dishes of the series (Fig. 4).

It shows that the fungus grows best on a medium that is slightly acid. At H-ion concentrations lower than pH 3.8 and higher than pH 7.0 the growth was retarded.

TABLE I.

Summary showing the amount of neutral potato agar used per tube, the number of drops of $\frac{n}{1}$ HCl or $\frac{n}{1}$ NaOH added to each and the reaction of each four days after preparation both in Fuller's scale readings and pH values.

Tube Number	Agar cc.	Drops of n/1 HCl	Drops of n/1NaOH	Dist. water in cc. added	Fuller's scale reading	pH values
1	10	6		5	+ 40	2.5
2	10	5		5	+ 30	3.0
3	10	4		5	+ 25	3.3
4	10	3		5	+ 20	3.8
5	10	2		5	+ 15	5.0
6	10	1		5	+ 10	6.2
7	10	0	0	5	+ 0.2	7.2
8	10		1	5	+ 0.3	8.0
9	10		2	5	+ 0.2	8.2
10	10		3	5	+ 0.3	8.4
11	10		4	5	+ 0.4	8.6
12	10		5	5	+ 0.4	8.8
13	10		6	5	+ 0.5	9.2

PATHOGENICITY

Isolation, Inoculation, and Reisolation

In inoculation experiments both in the field and in the greenhouse the fungus has been found repeatedly to be pathogenic on the leaf blades and leaf sheaths of oats. In inoculation experiments infection has not been obtained on the culm, panicle or mesocotyl.

The fungus was isolated from diseased areas on oat leaves by obtaining the pycnospores from the pycnidia, by placing the diseased area in distilled water for about fifteen minutes.

The surfaces of agar poured plates were inoculated with the spore suspension. After twelve hours germinating spores were observed with the aid of a microscope and marked for transfer. Single germinating spores were then transferred to slanted agar tubes. The surface of the slants were covered by the fungus in six to eight days. After six or seven weeks pycnidia and perithecia were formed in the test tubes. Mature pycnospores were obtained from the cultures and used suspended in

water as inoculum. Oat seedlings were inoculated with an atomizer and placed in a moist chamber for four days. These seedlings were dried every day and sprayed with distilled water and covered every

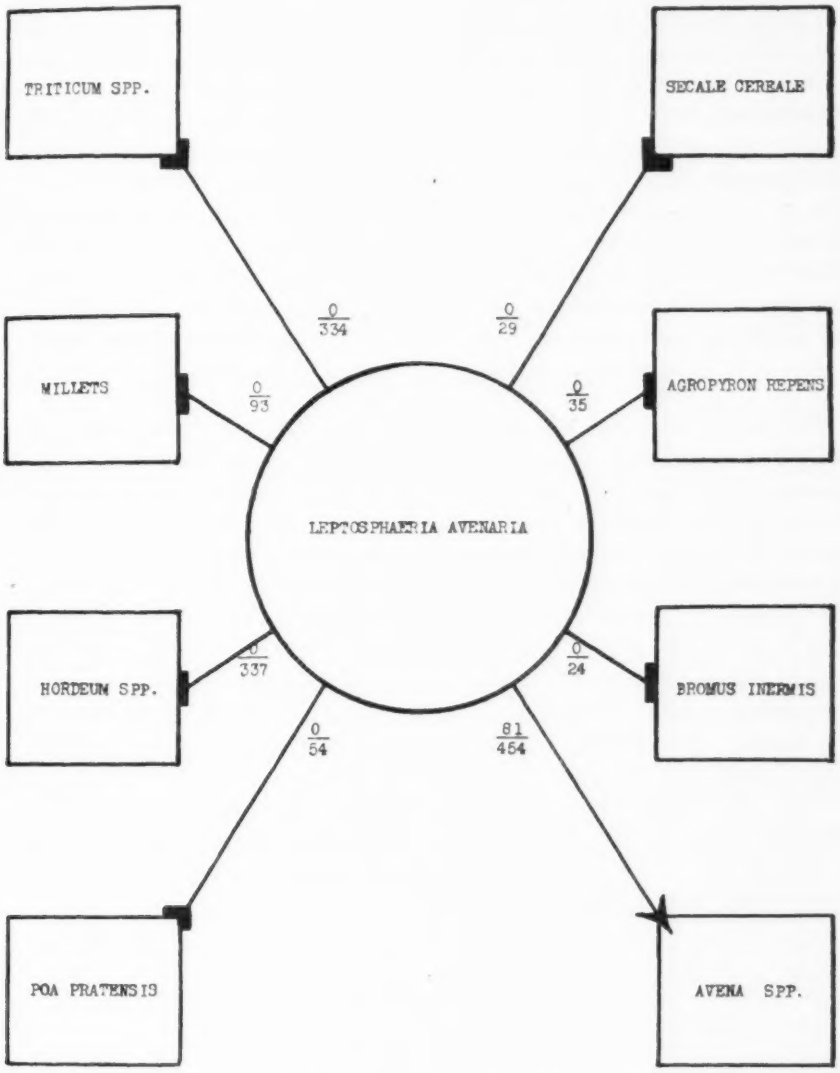

Fig. 5. Diagram summarizing results given in table 2. Inoculum from center circle, hosts inoculated in rectangles. Denominator of fraction designates number of leaves inoculated and numerator signifies the number which became infected.

night during the four days; they were then placed on a greenhouse bench. After fifteen days characteristic pycnidia were developed on the inoculated leaves while the uninoculated plants remained healthy. A parallel series of inoculations were made in which the inoculum was obtained directly from oat leaves collected in the field. Two weeks after inoculation pycnidia developed which were identical with those developed on the plants inoculated with pycnospores grown in culture. Reisolations were made from both series and the organism obtained, in the form of pycnospores, mycelium and pycnidia was identical with the organism from the original material. Inoculation experiments were also conducted in which the inoculum consisted of water suspensions of ascospores developed in culture. The inoculation methods were the same as previously described for inoculations with pycnospores. About two weeks after inoculation, infections were obtained on the leaves of oat seedlings. All species listed in table 2 were found susceptible.

Cross Inoculation Experiments

The disease was found in the field only on *Avena sativa*. Experiments have been conducted in an effort to determine the host range among the species of Avena, the other cereal crops and certain related grasses. In no case has infection been obtained on any host other than certain species of Avena. Among these species there is considerable variation as to susceptibility to the disease. The reproduction of the disease has been somewhat erratic. In certain experiments some species of Avena were susceptible and in other experiments identical results were not always obtained even when the same methods were used. Disturbed greenhouse conditions may have been responsible for some of the discrepancies. The results of the inoculation experiments are given in table 2 and figure 5.

From the results given in table 2 it will be noted that infections were obtained only on species of Avena, indicating distinct specialization to this genus. Certain species seem to be difficult to infect, especially *Avena fatua* and *A. strigosa*.

TABLE 2.

Summary of results from inoculating various grains and grasses with Leptosphaeria avenaria.

Hosts	Source of inoculum[1]	Number of leaves	
		Inoculated	Diseased
Avena barbata	1	27	8
	2	8	0
	3	6	1
A. brevis	1	30	3
	2	6	1
	3	8	0
A. fatua	1	27	0
	2	7	0
	3	9	7
A. nuda chinensis	1	22	2
	2	9	1
	3	5	1
A. sativa (Culbertson)	1	14	3
	2	7	0
	3	4	2
A. sativa (Swedish select)	1	20	4
	2	3	1
	3	6	0
A. sativa (Victory)	1	27	4
	2	9	2
	3	5	2
A. sativa nigra	1	18	6
	2	6	1
	3	6	4
A. sativa orientalis	1	22	4
	2	4	1
	3	5	0
A. sterilis (*a*)	1	15	3
	2	8	2
	3	4	0

[1] Source of inoculum as designated in table 2 is as follows: 1. Pycnospores from oats. 2. Pycnospores from culture. 3. Ascospores from culture.

Hosts	Source of inoculum[1]	Number of leaves	
		Inoculated	Diseased
A. sterilis (Rust Proof)	1	19	3
	2	4	1
	3	6	2
A. sterilis (b)	1	29	5
	2	7	0
	3	7	3
A. strigosa	1	25	0
	2	15	3
	3	7	0
Hordeum deficiens	1	17	0
	2	9	0
	3	5	0
H. horsfordianum (Ore.)	1	22	0
	2	7	0
	3	4	0
H. horsfordianum (S. Dak.)	1	27	0
	2	11	0
	3	6	0
H. jubatum	1	7	0
	2	5	0
	3	6	0
H. murinum	1	12	0
	2	9	0
	3	6	0
H. distichon erectum	1	7	0
	2	7	0
	3	4	0
H. distichon nudum	1	11	0
	2	6	0
	3	7	0
H. distichon nutans	1	5	0
	2	8	0
	3	6	0

Host	Source of inoculum[1]	Number of leaves	
		Inoculated	Diseased
H. vulgare coerulescens	1	12	0
	2	7	0
	3	3	0
H. v. hexastrichum	1	11	0
	2	4	0
	3	5	0
H. v. himalya	1	9	0
	2	5	0
	3	7	0
H. v. nigrum	1	8	0
	2	6	0
	3	9	0
H. v. pallidum	1	8	0
	2	6	0
	3	6	0
H. v. trifurcatum	1	10	0
	2	8	0
	3	7	0
Triticum aestivum	1	19	0
	2	5	0
	3	4	0
T. a. lutescens (Harvest Queen)	1	19	0
	2	4	0
	3	6	0
T. a. miltura (Red Wave)	1	7	0
	2	6	0
	3	6	0
T. compactum	1	19	0
	2	6	0
	3	7	0
T. dicoccum barrum	1	18	0
	2	7	0
	3	7	0

Hosts	Source of inoculum[1]	Number of leaves	
		Inoculated	Diseased
T. d. atratum	1	15	0
	2	4	0
	3	5	0
T. durum	1	24	0
	2	7	0
	3	4	0
T. monococcum	1	24	0
	2	7	0
	3	4	0
T. polonicum	1	20	0
	2	5	0
	3	6	0
T. spelta	1	19	0
	2	5	0
	3	2	0
T. turgidum	1	16	0
	2	3	0
	3	7	0
Secale cereale	1	18	0
	2	6	0
	3	3	0
Agropyron repens	1	16	0
	2	11	0
	3	8	0
Bromus inermis	1	11	0
	2	6	0
	3	7	0
Chaetochloa italica	1	5	0
	2	4	0
	3	8	0
C. viridis	1	9	0
	2	6	0
	3	4	0

Hosts	Source of inoculum[1]	Number of leaves	
		Inoculated	Diseased
Echinochloa crus-galli	1	7	0
	2	3	0
	3	5	0
Panicum clandestinum	1	13	0
	2	5	0
	3	7	0
Pennisetum glaucum	1	6	0
	2	3	0
	3	6	0
Poa pratensis	1	32	0
	2	10	0
	3	12	0

MODE OF OVERWINTERING

The pycnospores remain viable over winter when retained in the pycnidia. A number of oat leaves containing pycnidia, were collected in October, 1921, tied together with cord and fastened to a stake in the field. Every two weeks a portion of the diseased leaf was taken to the laboratory and the spores tested for percentage of germination. In every case except the collection made January 16, 1922, 90 per cent germination or above was found. On January 16, 77 per cent of the spores germinated.

VIABILITY AND LONGEVITY OF SPORES

Pycnospores were collected in the field in September, 1921; at that time 100 per cent of the spores germinated. Some of the diseased material was placed out of doors and on April 1, 1922, 90 per cent of the spores germinated. Some of the material was kept in a paper envelope in the laboratory. From this material 58 per cent of the spores germinated on April 1, 1922.

MODE OF INFECTION

Seedlings for these studies were grown in the greenhouse in pots until they were about six inches high. They were then inoculated by atomizing one series with a water suspension of pycnospores and the other with a water suspension of ascospores. Following these inoculations the plants were placed in a moist chamber for three or four days and then removed

to the greenhouse. Every 12 hours after inoculation for a six day period leaf samples were collected and placed in vials containing equal parts of 95 per cent alcohol and glacial ascetic acid. They were left in this solution for from 24–36 hours, which removed the chlorophyll leaving the leaf tissue almost white. These pieces were then stained in toto with Pianeze IIIb stain according to the method given by Vaughan (8). As the result of staining and clearing, the leaf tissues were light bluish-green in color and almost transparent, and the spores and mycelium were a rose red (Pl. XXX). The mycelium could be readily followed over the surface of the epidermal cells as well as in the parenchymatous tissue between the epidermal layers.

It was found that both pycnospores and ascospores behaved similarly in infection, namely, they lodge in the furrows between the epidermal cells and there develop germination tubes, which grow over the leaf surface. The tips apply themselves to the cuticle directly above adjoining walls of epidermal cells. The infecting hyphae penetrate the cuticle and grow between the epidermal cells and then continue between the parenchymatous cells branching in various directions. Pycnidia developed below the stomata by the hyphae collecting and matting together.

PERIOD OF INCUBATION

The time required for the reproduction of the disease, from inoculation to mature pycnospores was from 12 to 16 days, averaging 14 days. The first evidence of infection appeared after 8 to 9 days, when a more or less mottling of the leaves became evident. Mature pycnidia were developed after about two weeks.

PATHOLOGICAL ANATOMY

The preparation of the oat leaves for the study of the pathological anatomy involved the methods previously described under "Mode of infection" except that Pianeze IIIb stain was diluted with 25 per cent alcohol to one fourth strength, and the leaf tissues were left in the stain for from eighteen to thirty hours. This stained the fungus a deeper red without proportionately darkening the host tissue. It was found that when the first symptoms appeared on the leaf eight or nine days after inoculation, the fungus had extensively invaded the intercellular spaces. Strands or hyphae growing just under the epidermis had extended to the length of several epidermal cells. Sometimes it was straight but more often slightly undulating or more or less sharply bent around the parenchymatous cells. These long strands were much branched and the

branches grew in different directions between the cells limited more or less by the vascular strands of the host. The branches that extended deeper into the host tissue grew irregularly often almost completely around a single host cell. Several hyphae growing in this manner readily fill the intercellular spaces. The larger more vigorous strands of hyphae more often extended lengthwise of the leaf than crosswise or directly into the tissue. No haustoria were found. At this stage none of the cells were killed. At the end of about two weeks pycnidia were found under the stomata. They were usually directly below the stomata although occasionally they were found to be a little to one side, and always with the ostiole directly under the stomatal aperture. The pycnidia occupy all of the sub-stomatal chamber and the larger pycnidia crowd the adjoining parenchymatous cells out of place. The pycnidia were surrounded by dense mats of mycelium involving not uncommonly several adjoining parenchymatous cells. The pycnidia were in rows conforming to the rows of stomata. The invading hyphae were usually the length of four to six epidermal cells in advance of the youngest mature pycnidia. All stages in the formation of pycnidia were found, from a few clustered hyphae to the large dense mature structures. The host cells close to the pycnidia were dead and collapsed. Host cells farther away were plasmolized but still held their form while those about which only a few hyphae were growing appeared to be unchanged.

SUMMARY

1. The speckled blotch of oats previously reported on *Avena sativa* in France, Germany and England was found to occur to a limited extent in Wisconsin.

2. It was found only on volunteer oats, hence it was of no economic importance. The symptoms are characteristic only after pycnidia develop.

3. The ascigerous stage of the fungus has been found and belongs to the genus Leptosphaeria. Accordingly this species is referred to that genus and given the name *Leptosphaeria avenaria*.

4. Pycnospores developed both on leaves of oats and in culture, and ascospores developed in culture germinated in water and on potato-dextrose agar after a few hours.

5. The cardinal temperatures for mycelial growth on agar poured plates are as follows: minimum 2° C., optimum 20 to 25° C., maximum 32° C.

6. In artificial culture the fungus grows best on an acid medium.

7. The fungus enters the host by direct penetration of the cuticle by germination tubes of both pycnospores and ascospores and then grows between the epidermal cells.

8. The period of incubation is from 12 to 16 days, the first symptoms appearing after eight days.

9. The mycelium grows intercellularly and the pycnidia are subepidermal.

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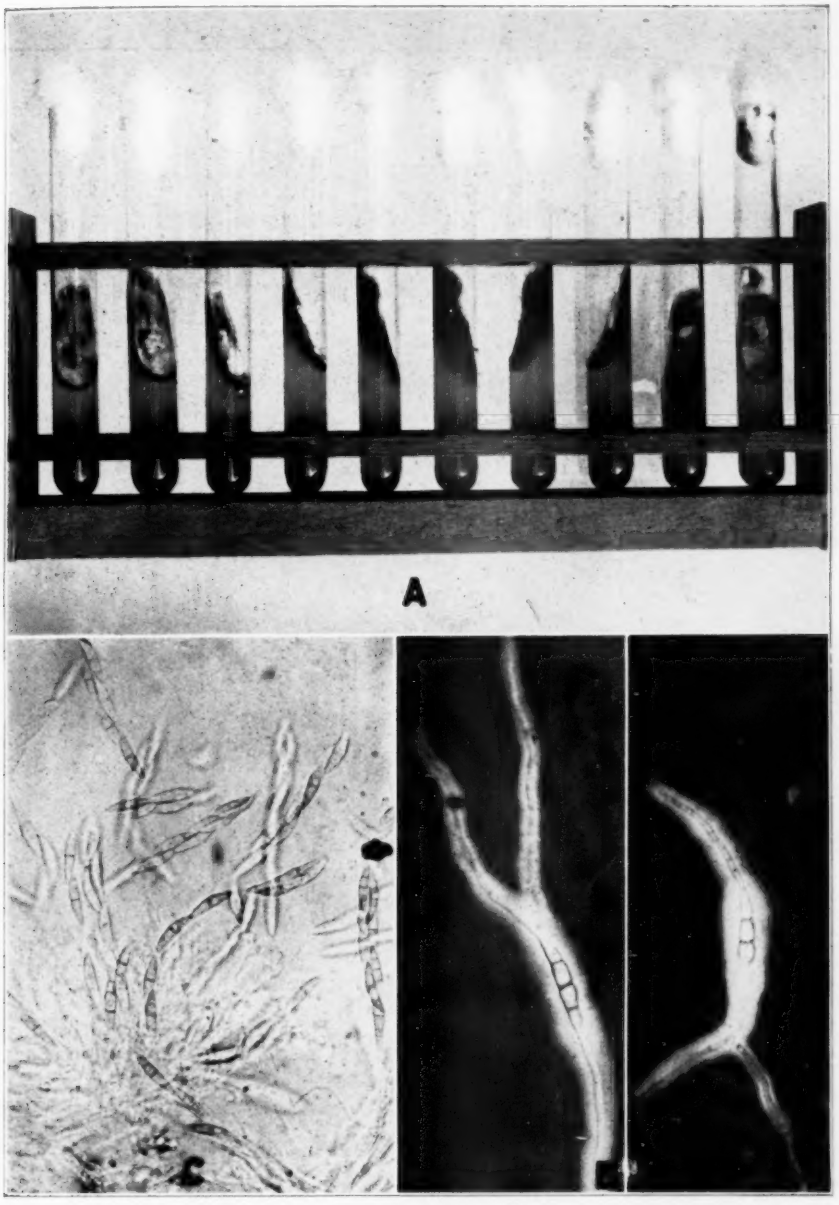

SPECKLED BLOTCH OF OATS

A. Potato-dextrose agar cultures in each of which pycnospores and ascospores developed. B. Photomicrographs showing germinated ascospores of *Leptosphaeria avenaria* in a medium of dilute India ink. Observe the transparent gelatinous sheath surrounding each spore and the older hyphae tapering toward the tips of the growing hyphae. × 900. C. Photomicrograph of ascospores showing general form and characteristic habit of adhering together after coming out of the ascus. × 250.

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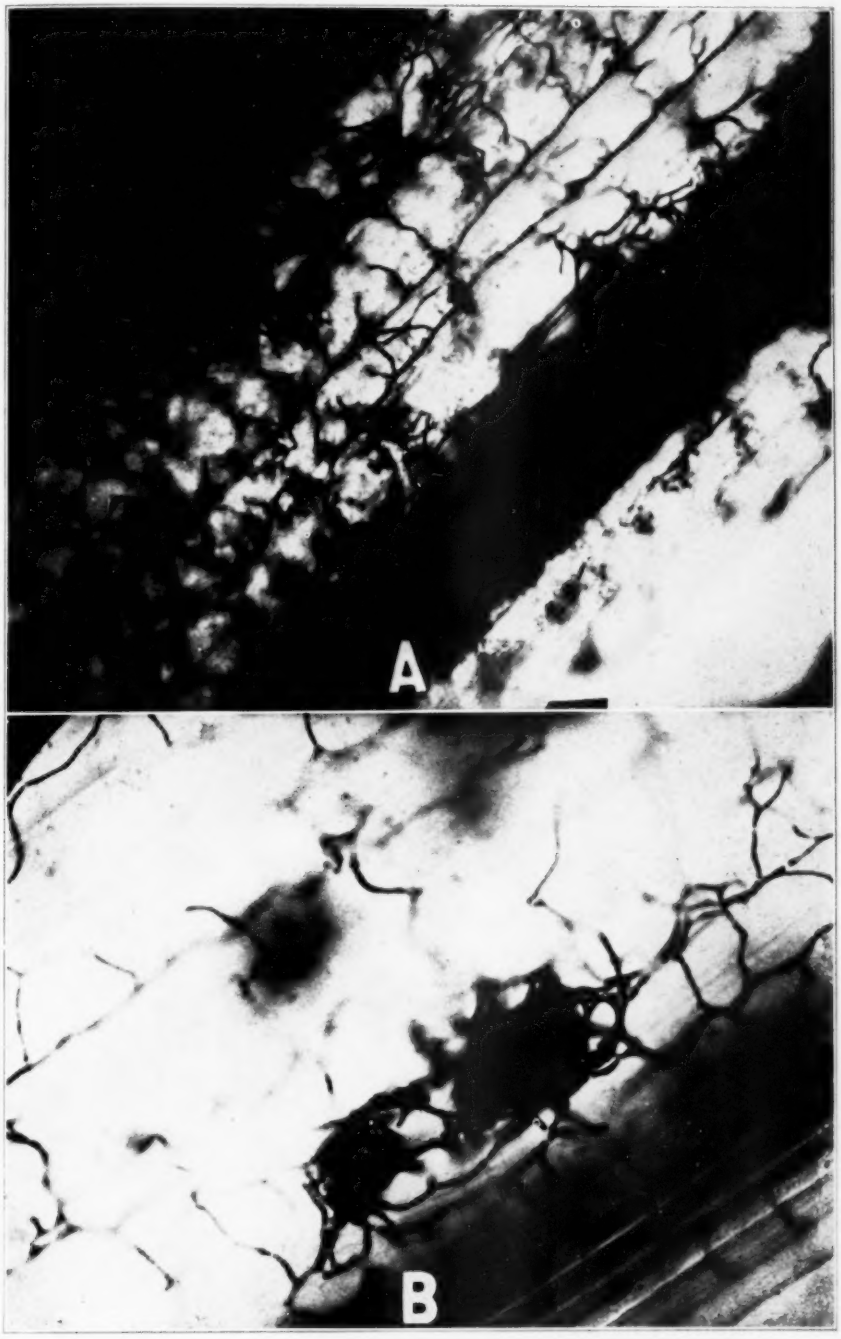

SPECKLED BLOTCH OF OATS

A. Photomicrograph showing hyphae of *Leptosphaeria avenaria* in the parenchymatous tissue of an oat leaf. x 400. B. Photomicrograph showing early stage in the formation of a pycnidium of *Leptosphaeria avenaria*. × 900.

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GERMINATION OF TELIOSPORES OF RUSTS AT COLUMBIA, MISSOURI.

W. E. Maneval

The teliospores of rusts generally germinate only after a more or less definite resting period, usually extending from summer or autumn of one year to the time of leaf development of the host the following year. The period of rest may be quite short, while there are numerous lepto-types and some others that require no resting period. Plowright (18) indicated what is true in this respect for many of the species listed in his monograph. Dietel (3) has published a rather long list of lepto-types and of some others whose teliospores germinate soon after maturity. These include eu-, brachy-, and hemi-types. He states that lepto-types are rare on Gramineae, Cyperaceae and Umbelliferae. Klebahn (13) says it is generally necessary for the teliospores of heteroecious rusts to pass the winter in the open. Eriksson (8) succeeded in germinating teliospores of *Puccinia graminis* after keeping them one or two winters in an herbarium and then one winter in the open. Woronin (24) found that teliospores of *Puccinia helianthi* would germinate equally well after passing the winter in the open or dry indoors, while Carleton (2) discovered that they would germinate to some extent in October without passing through a resting period. Klebahn (13, p. 12) divides heteroecious rusts into 6 developmental types. In one type the teliospores germinate only after a winter rest period (most species of Uromyces Puccinia, Melampsora, and Pucciniastrum), while the other 5 types include forms that hibernate as aecial or telial mycelium or whose method of hiberation is unknown. In these 5 types the teliospores germinate immediately after maturity.

During the past 5 years the writer has made observations at various dates between fall and spring on the germination of teliospores of a number of species of rusts occurring at Columbia, Missouri. The following species were used in the germination tests:

Phragmidium potentillae-canadensis Diet. on *Potentilla canadensis* L.

Puccinia asparagi DC. on *Asparagus officinalis* L.

Puccinia helianthi Schw. on species of Helianthus, mainly *Helianthus strumosus* L. and *Helianthus hirsutus* Raf.

Puccinia menthae Pers. var. *americana* Burr. on *Monarda fistulosa* L.

Puccinia ruelliae (B. & Br.) Lagh. on *Ruellia strepens* L.

Puccinia andropogonis Schw. on *Andropogon furcatus* Muhl.

Puccinia peridermiospora (Ell. & Tracy) Arth. on *Spartina michauxiana* Hitch.

Puccinia sorghi Schw. on *Zea mays* L.

Puccinia sydowiana Diet. on *Sporobolus asper* (Michx.) Kunth.

Puccinia windsoriae Schw. on *Tridens flavus* (L.) Hitch.

The tests show that all of these forms may germinate at the beginning of winter (December) and in some cases earlier. These rusts are all eu-types. The first 5 are autoecious and the last 5 heteroecious. The data presented serve as records of germination of these rusts at a definite station and have a bearing on conditions for germination. The records for 4 of the species are for one year only, while in the other cases they are for two to four years. Some of the factors especially concerned in the germination of teliospores of rusts are moisture, temperature, oxygen supply, and maturity or age of the spores, while light, substratum and certain other conditions seem to be of minor importance. Some effects of these factors will be briefly reviewed.

Moisture. Generally a practically saturated atmosphere is necessary for germination. The amount of moisture present influences germination, morphology and production of sporidia. Blackman (1) found that teliospores of *Puccinia graminis, Uromyces fabae* and *Phragmidium rubi* immersed in water developed long promycelia but no sporidia until they grew out into the air; however if not immersed they produced characteristic promycelia and sporidia. Weimer (23) also got long promycelia of *Gymnosporangium juniperi-virginianae* in water, but if little moisture was present the usual type of promycelia bearing sporidia developed. Melhus, Durrell and Kirby (17) found that teliospores of *Puccinia graminis* attached to pieces of straw would not germinate if the humidity of the atmosphere was less than 96.5 per cent.

The effect of drying and especially of alternate wetting (soaking) and drying of teliospores has been noted repeatedly. Dietel (4, vol. 31) found that drying decreased markedly the time (from three days to two and three-fourths hours) necessary for germination to begin in the case of *Melampsora larici-caprearum* Kleb. In other experiments (4, vol. 54) he collected teliospores of the same rust October 31, 1915, and kept them dry until November 4 and 13. On these dates he subjected the spores to alternate wetting and drying for periods of two or three days until December 21, when they would all germinate, there being no difference between the two sets. In 1916 spores of this rust were collected November 11 and after three soakings germinated abundantly December 16. The same result was secured in 1917, with spores collected November 22, and soaked five to seven times. Dietel concludes that teliospores

are not in an unchangeable condition and that germination may be brought about earlier than normally by alternate wetting and drying.

In certain germination tests in 1911, with *Puccinia graminis* Pers. on *Agropyron repens* P. De Beauv., *Puccinia phragmites* (Schum.) Korn. on *Phragmites communis* Trin. and *Puccinia magnusiana* Korn. on *Phragmites communis*, Klebahn (14) found that teliospores kept dry in the open, exposed to the cold and dampness of the air but not rain, did not germinate May 1. Tests resulted negatively also with spores kept in a dry room over winter; but spores of *Puccinia graminis* and *Puccinia phragmites* kept alternately wet and dry (three day periods) from November 7, to April 24 ,with the temperature always above 0° C. germinated abundantly May 1.

Similar results were obtained by Klebahn in 1912, after subjecting spores to flowing water and to alternate wetting and drying for three and eight day periods. The experiments were started with *Puccinia phragmites* November 13, and with *Puccinia graminis* November 29. Spores kept alternately wet and dry and tested January 10 germinated quite well. Controls of *Puccinia graminis* kept in a warm room germinated slightly while those of *Puccinia phragmites* did not germinate. Klebahn concludes that the most important factor for germination is alternate saturation with water and drying. Changing the water proved important also and this, he suggests, may be in connection with the oxygen content. Winter cold was not necessary, but seemed to have an accelerating influence on germination.

Temperature. Many more records are available regarding the relation of temperature to germination of urediniospores than of teliospores. The work of Johnson (12), Ward (22), Gibson (10), Mains (16), Doran (5), Howell (11) and Durrell (6) shows that for nearly twenty species of rusts the cardinal temperatures for germination of urediniospores are almost without exception between the following limits: minimum 2° C. to less than 10° C.; optimum, 12° C. to 20° C.; maximum, 25° C. to less than 30° C. Compared with cardinal temperatures for germination of spores of other fungi these are rather low, and it seems that the same holds true for aeciospores and teliospores. Doran (5) reports 5°, 12° and 19° C. as cardinal temperatures for germination of aeciospores of *Cronartium ribicola* and 9°, 16° and 29° C. for aeciospores of *Gymnosporangium clavipes*.

Dietel (4, vol. 35) states that he found no influence of temperature on germination of teliospores of *Melampsora larici-caprearum* between 8° C. and 22° C. and secured good germination even at 26° C. He concluded for *Puccinia graminis* that the minimum temperature for

germination is probably about 9.5° C., and normal germination occurred at 22° C. At 23° C. and higher the promycelia were abnormal and produced neither sterigmata nor sporidia. Others have reported cardinal temperatures for germination of teliospores of rusts as follows:

Puccinia malvacearum	3°	14°	30° C.	Doran (5)
Puccinia graminis	5°	20°	30° C.	Melhus (17) No sporidia above 25° C.
Puccinia helianthi	20—°	24°±	28+° C.	Fraizer (9)
Gymnosporangium juniperi-virginianae	11.5°	15°	29° C.	Reed & Crabill (19) No sporidia above 24° C.
Gymnosporangium juniperi-virginianae	7°	22–25°	29° C.	Weimer (23)
Gymnosporangium globosum	7°	22–25°	29° C.	Weimer (23)
Gymnosporangium clavipes	7°	22–25°	29° C.	Weimer (23) Some sporidia at 26° C.

These temperatures correspond in general with the cardinal temperatures for germination of urediniospores and aeciospores. The germination of spores of various fungi is favored by exposure to low temperature, but that this is unnecessary for germination of teliospores of various rusts has been demonstrated repeatedly. McAlpine (15) kept samples of teliospores of *Puccinia graminis* on badly rusted straw at 4° C. and—18° C. for three months and also in the open in Australia. When tested in the spring only those kept in the open germinated (p. 24). He also states (p. 66) that, "seeing that the spores (teliospores of *Puccinia graminis*) germinated freely here, the exposure to cold theory does not hold." He also says (p. 9) that germination tests in Victoria during several seasons resulted positively only from September to November (spring) after a resting period of about eight months including the hot and dry summer.

Chemicals. No one, it seems, has reported improvement in germination of teliospores by using complex nutrient solutions. It is likely that oxygen is absolutely necessary for germination. Reed and Crabill (19) failed to secure germination of *Gymnosporangium juniperi-virginianae* in an atmosphere of carbon dioxide and state that oxygen is apparently essential. Dietel (4, vol. 54) tested teliospores of *Puccinia graminis*, *Melampsora larici-caprearum* and *Melampsora larici-tremulae* in an atmosphere of carbon dioxide, in air with the oxygen removed, and in a vacuum. When kept under such conditions for 4, 24 and 48 hours, the spores did not germinate. These spores were then put in air and germination occurred in every case as well as in all controls.

Thiel and Weiss (21) treated telial material of *Puccinia graminis*

collected August 12, 1919, in Minnesota with 1 per cent citric acid for five minutes to one hour and on testing secured from one to fifteen per cent of germination with spores treated fifteen or twenty minutes. The spores were exposed to out-door conditions until the time of testing (December 23 and 29; January 14 and 26; February 21), but there was little difference in the percentage of germination on these different dates. Treatments with m/100 HCl, m/100 to m/5 H_2SO_4, 1 per cent H_3PO_4, 1, 2.5 and 5 per cent lactic and acetic acids, 0.0625 to 5 per cent H_2O_2, etc., resulted negatively. It seems possible that with the acid treatments in most cases the concentration may have been too high to hope for a favorable influence.

Maturity. If we regard the changes that occur in teliospores during the winter as a maturing process this may explain in a way several phenomena connected with germination. All of the spores in a pustule do not germinate at one time but during a considerable period of time. Reed and Crabill (19) found that teliospores of *Gymnosporangium juniperi-virginianae* germinated after every considerable rain from April 1 to June 1, those on the outside of the tentacle germinating first. McAlpine (15) makes similar statements regarding *Puccinia graminis* (p. 9 & 24). Eriksson (7) was of the opinion that only the crop of teliospores of *Puccinia graminis* maturing in late autumn is able to germinate the following spring. As spring approaches the percentage of spores that will germinate in a given species requiring a rest period increases gradually to a maximum and then declines. Moreover, it has been noted by certain observers that as the time of maximum germination approaches the time necessary for germination to start decreases, sometimes to an hour or less. Dietel (4, vol. 35) germinated the teliospores of *Puccinia graminis* at the middle of March in central Germany and found that the time necessary for germination to begin decreased gradually from thirty hours to two or two and three-fourths hours, and then increased until practically no more germination occurred July 1. He found also that spores of *Puccinia malvacearum* began to germinate in fifteen minutes, the formation of sporidia began in two hours, and in some cases was complete in two and one-half hours.

EXPERIMENTAL

After collecting telial material it was kept at room temperature (20°± C.) or in a few cases, as stated below, in a cold room somewhat above 0° C. In making germination tests the teliospores were floated on the surface of distilled water in a preparation dish of 30 cc. capacity. The amount of water used varied between 10 and 15 cc. The dishes were

kept covered and at room temperature (20±° C.). It was difficult to determine accurately the percentage of germination so generally the amount of germination is indicated approximately as follows: 0 = none; + = few spores to less than 1 per cent germinating; ++ = 1–5 per cent; +++ = 10 ± per cent or good; ++++ = 30–90 per cent or very good to excellent. Wherever approximate percentages are given they were obtained by removing carefully a part of the spore suspension to a drop of water on a slide and then counting the number of germinated and ungerminated spores in several fields of the microscope. Most of the tests were made in duplicate and the cultures were examined every day or more frequently for several days, and after that at intervals of two or three days. Examinations for germination could readily be made with the low power of the microscope without removing spores from the culture dish.

In the tables that follow the principal results of the tests are given. With certain forms, particularly *Puccinia helianthi*, *Puccinia peridermiospora* and *Puccinia windsoriae* many more tests were made than are presented in detail here.

PUCCINIA ASPARAGI

Only one test was made with this rust. Spores collected December 27, 1921, and tested the same day began to germinate in 6 days or less, and during the next 10 days approximately 5 per cent germinated. Germination in m/15 KH_2PO_4 (pH 4.6)[1] and in m/15 KH_2PO_4 plus m/15 K_2HPO_4 (pH 5.4) was decidedly poorer than in water.

Smith (20) did not succeed in germinating teliospores of this rust in California betweeen June 20 and December 12, using material collected between June 8 and November 19. He did succeed, however, in germinating a few spores collected December 1 and tested December 12. The germinating capacity increased as the season advanced to February 26 (last test).

PUCCINIA SYDOWIANA

In a test of spores collected December 27, 1921, and started two days later germinating spores were observed at the end of 22 days and the percentage of germination increased to between 2 and 5 per cent in the next 3 weeks.

Spores of the same collection were also frozen in ice the night of January 6, and then kept dry in a cold room until January 13. They

[1] Hydrogen-ion concentration in all cases was determined colorimetrically by Gillespie's method.

were then frozen in ice for four successive nights, January 13 to 16, after which they were dried and tested January 18. In this test the time for germination to begin was decreased and in 3 weeks a higher percentage (5 to 10 per cent) had germinated than during 6 weeks in the preceding test.

PUCCINIA SORGHI

This rust was collected December 27, 1921, and tested December 29. Germination began in less than 2 days and increased from day to day until most of the spores had germinated at the end of 11 days.

Another test was made January 13, with spores from the same collection as used in the previous one. The spores had been kept in a cold room in the meantime and germinated as well or better than when tested soon after collection.

Germination was retarded in m/15 KH_2PO_4 (pH 4.6) and in a mixture of m/15 KH_2PO_4 and m/15 K_2HPO_4 (pH 5.4) during the early part of the germination period, but finally (11 days) as large a percentage of spores had germinated as in water. These facts are summarized in table 1.

Mains (16) states that the teliospores of *Puccinia sorghi* require wintering before germinating. However, he collected fresh material in the greenhouse in January and after alternate soaking and drying for 3 day periods repeated 5 times secured fair germination.

TABLE 1

Germination of teliospores of Puccinia sorghi

Collected	Tested	Incubation period	Result	Remarks
12/27/21	12/29/21	2 days	5–10 per cent	
"	"	5 "	50± per cent	
"	"	11 "	90± per cent	
"	"	5 "	20–30 per cent	In phosphates, pH 4.6 and 5.4.
"	"	11 "	90± per cent	In phosphates, pH 4.6 and 5.4.
"	1/13/22	3 "	50± per cent	Had been kept in cold room.

PUCCINIA RUELLIAE

With this rust it will be noted (Tab. 2) that the time required for considerable germination of the spores varies with the date of testing, being less than 2 days in May, less than 8 days in December and decidedly longer in September. Also, as with the preceding species, the percentage of germination may increase gradually over a considerable period of

time, as is seen in the test begun September 13, 1918, in which a very high percentage of spores germinated in 53 days.

TABLE 2

Germination of teliospores of Puccinia ruelliae

Collected	Tested	Incubation period	Result	Remarks
10/23/16[1]	4/5/17	5 days	0	
"	"	8 "	+ +	
"	5/7/17	43 hours	+ + +	
9/10/18	9/13/18	39 days	+ + +	
"	"	53 "	+ + + +	
"	10/ 3/21	32 "	+ + +	Wet and dry, 3 day periods, 9/13 to 10/3.
12/27/21	12/29/21	8 "	5 per cent	

Acid reaction (phosphates, pH 4.6 and 5.4) retarded germination as was the case with *Puccinia sorghi* and *Puccinia asparagi*.

PHRAGMIDIUM POTENTILLAE CANADENSIS

Only two collections of this rust, made in the fall of 1917, were tested. Germination of the spores in each case began in from 2 to 5 days and was practically complete in 20 days in the later collection and in 28 days in the earlier one. Again after germination began (2 to 5 days) it extended over periods of 3 to 4 weeks.

TABLE 3

Germination of teliospores af Phragmidium potentillae canadensis.

Collected	Tested	Incubation period	Result
10/7/17	11/3/17	5 days	+
"	"	12 "	+ +
"	"	28 "	100— per cent
10/16/17	10/19/17	15 "	+ + +
"	"	20 "	100— per cent
"	11/3/17	2 "	+
"	"	7 "	+ +

PUCCINIA MENTHAE VAR. AMERICANA

Teliospores collected November 22, 1921, germinated fairly well in 15 days, and approximately fifty per cent from the collection of December 27, germinated in 3 days. This time was still shorter (1½ days) in

[1] All rusts collected October 23, 1916, were put in muslin bags and kept out of door during the winter and were partially protected from rain and snow.

tests made March 10, 1920. Two tests with solutions of phosphates (pH 4.6 and 5,4), using spores collected December 27, 1921, resulted in very marked inhibition of growth during the first 3 days of the test, with considerable inhibition in the less acid solution after 6 days and marked inhibition in the more acid one. It was found, also, that this rust and certain others might be kept floating on water at temperatures above the maximum for germination for 2 or 3 days and then, if changed to a temperature near the optimum, they would germinate.

TABLE 4

Germination of teliospores of Puccinia menthae var. americana

Collected	Tested	Incubation period		Result	Remarks
10/23/16	4/5/17	3	days	+ + + +	
"	4/16/17	67	hours	0	28° C.; then 25 hours longer at room temperature + + +.
1/5/18	1/7/18	3	days	+ + + +	
3/9/20	3/10/20	1½	"	+ + + +	
11/22/21	11/22/21	15	"	+ + +	
12/27/21	12/27/21	6	"	80 per cent	
"	12/29/21	2	"	20–30 per cent	3 days, 50± per cent
"	12/27/21	3	"	5—per cent	m/15 phosphate, pH 4.6 and 5.4 per cent
"	"	6	"	10 per cent	m/15 phoshates, pH 4.6; 50 ± per cent with pH 5.4

PUCCINIA WINDSORIAE

Teliospores of this rust collected December 17, 1918, germinated somewhat in 35 days and those collected December 27, 1921, germinated slightly in 10 days while 5 ± per cent germinated in 41 days. Similar results were obtained with material collected January 5, 1918, and January 9, 1922. Spores collected October 16, 1917, failed to germinate in 61 days, but when kept till January and then subjected to alternate wetting and drying for two weeks they germinated slightly in 17 days. The time for germination however, decreased to two days for spores collected in April, 1918 and 1922.

The percentage of spore germination with this rust was generally not high. Alternate wetting and drying in the case of collections made January 15, 1918, and February 26, 1918, seemed to improve the germination and to shorten the time necessary for it to begin. Spores collected April 30, 1918, did not germinate in two days at either 28° C. or 32° C. although they did germinate to some extent when kept two

days longer at room temperature. Most of the facts mentioned are summarized in table 5.

TABLE 5

Germination of teliospores of Puccinia windsoriae

Collected	Tested	Incubation period	Result	Remarks
2/26/18	2/27/18	12 days	0	
"	3/7/18	4 "	0	1 day wet, 3 days dry, 2/27–3/7
"	3/11/18	4 "	+	1 day wet, 3 days dry, 2/27–3/11
"	3/15/18	2 "	+ +	1 day wet, 3 days dry, 2/27–3/15
4/30/18	4/30/18	2 "	+ +	Had germinated in part out of doors
10/16/17	10/16/17	61 "	0	
"	1/28/18	17 "	+	3 days wet, 3 days dry, 1/7 to1/28
"	2/23/18	9 "	+ +	3 days wet, 3 days dry, 1/7 to2/23
9/10/18	9/13/18	36 "	0	
12/17/18	12/17/18	35 "	+ + +	
1/5/18	1/7/18	31 "	+	Much better in 39 days
"	1/28/18	21 "	+ +	3 days wet, 3 days dry, 1/7 to 1/28
"	1/30/18	8 "	+ +	1 day wet, 2 days dry, 1/15 to 1/30
"	2/11/18	3 "	+ + + +	1 " wet, 2 " dry, 1/15 to 2/11
12/27/21	12/29/21	10 "	+	5± per cent in 41 days.

PUCCINIA ANDROPOGONIS

Alternate wetting and drying improved the germination of this rust. Spores from collection made December 17, 1918, failed to germinate floating on water in 35 days, but they germinated quite well in 6 days after alternate wetting and drying from December 17 to January 26 (3 day periods). Spores collected January 5, 1918, also failed to germinate in water in 39 days but two cultures germinated somewhat in 3 and 7 days after alternate wetting and drying from January 7 to February 11 and 13 respectively. Also teliospores collected February 26, 1918, did not germinate in water in 12 days but when kept alternately wet and dry (1 day wet and 3 dry) and tested March 7, 11 and 15 some spores germinated in 1 or 2 days and in the case of the last test 20 to 25 per cent germinated in 3 days (Table 6).

That teliospores of this rust may germinate as early as December was determined by tests made with material collected December 27, 1921, and tested December 29, when numerous spores germinated in 2 days. However very few more spores germinated in these cultures when kept 16 days longer. Spores of this same collection were frozen in ice January 6, dried, soaked in water January 9, dried and tested January 10. In the course of 3 days about 5 per cent germinated.

TABLE 6

Germination of teliospores of Puccinia andropogonis

Collected	Tested	Incubation period	Result	Remarks.
12/17/18	12/17/18	35 days	0	
"	1/26/19	6 "	+ + +	3 days wet, 3 days dry, 12/17 to 1/26.
1/5/18	1/7/18	39 "	0	
"	1/28/18	17 "	0	3 days wet, 3 days dry, 1/7 to 1/28.
"	2/11/18	3 "	+	3 days wet, 3 days dry, 1/7 to 2/11
1/15/18	1/30/18	15 "	0	1 day wet, 2 days dry, 1/15 to 1/30.
"	2/11/18	3-14 "	+	1 day wet, 2 days dry, 1/15 to 2/11.
2/26/18	2/27/18	12 "	0	
"	3/7/18	2-6 "	+	1 day wet, 3 days dry, 2/26 to 3/7.
"	3/11/18	4 "	5 per cent	1 day wet, 3 days dry, 2/26 to 3/11.
"	3/15/18	3 "	20-25 per cent	1 day wet, 3 days dry, 2/26 to 3/15.
3/9/20	3/10/20	23 "	0	
"	4/8/20	2-4 "	+ +	
12/27/21	12/29/21	2, 5, 18 "	+	
12/28/21	1/10/22	3 "	5 per cent	Frozen in ice 1/6; soaked 1/9.

PUCCINIA PERIDERMIOSPORA

Teliospores of this rust collected October 16, 1917, and September 28, 1918, failed to germinate in 61 and 38 days respectively. Spores collected December 17, 1918, and January 5, 1918, germinated quite well after 35 and 32 days. The time for germination to begin or to reach a high percentage gradually decreased between December and April as with other rusts. This is seen too on comparing dates much closer together; for example, while the collection of March 19, 1917, required 10 days for slight germination considerable germination occurred in the collection of April 6, 1917, in 2 days. The effect of alternate wetting and drying is illustrated by the collection of February 26, 1918, which required 10 days for slight germination but germinated very well (40± per cent) in from 2 to 5 days after alternate wetting and drying between February 27 and March 20. It seems too that considerable change may occur even though the spores are kept dry at room temperature, for while the spores collected March 9, 1920, germinated somewhat after 15 days those tested April 8 germinated very well in 6 days. This is also illustrated by the behavior of other species. Spores of *Puccinia andropogonis* collected March 9, and tested March 10, 1920, failed to germinate in 23 days but some germination occurred in from 2 to 4 days when the spores were tested April 8.

TABLE 7.

Germination of teliospores of Puccinia peridermiospora

Collected	Tested	Incubation period	Result	Remarks
3/19/17	3/20/17	10 days	+	
4/6/17	4/7/17	2 "	+ + +	
5/5/17	5/7/17	3 "	0	Apparently germinated out-doors.
12/17/18	12/17/18	35 "	+ + + +	Not examined till 1/21/19.
"	1/26/19	6 "	+ + +	3 days wet, 3 days dry, 12/17 to1/26.
1/5/18	1/7/18	32 "	+ + +	
"	1/28/18	10 "	+ +	3 days wet, 3 days dry, 1/7 to 1/28.
"	2/11/18	2–5 "	+ +	3 days wet, 3 days dry, 1/7 to 2/11.
1/15/18	1/30/18	8 "	+ + +	1 day wet, 2 days dry, 1/15 to 1/30.
2/26/18	2/27/18	10 "	+	
"	3/11/18	1 "	+ +	1 day wet, 3 days dry, 2/27 to 3/11.
"	3/20/18	2 "	+ + +	1 day wet, 3 days dry, 2/27 to 3/20.
"	"	5 "	40 ± per cent	1 day wet, 3 days dry, 2/27 to 3/20.
3/13/18	3/13/18	5 "	0	
3/9/20	3/10/20	15 "	+ +	
"	4/8/20	6 "	+ + + +	
4/30/18	4/30/18	1 "	+ +	
12/27/21	12/27/21	18 "	0	
12/28/21	2/7/22	13 "	10 ± per cent	20 days, 100— per cent in good part.

PUCCINIA HELIANTHI

Woronin (24) collected teliospores of this rust in the fall (date not given) and kept them wrapped in paper in a room. Nearly 100 per cent germinated in 2 days, February 3, and spores tested between February and the middle of May germinated very well, regardless of preservation at room temperature or covered with snow all winter. Gradually the power to germinate was lost so that tests made July 19, resulted negatively at the end of 3 days, and only a few spores germinated in 5 days.

Many tests were made with *Puccinia helianthi* but most of the results were similar to those given in table 8. Spores collected in October would germinate slightly in two months or more. Spores collected October 7, 1917, germinated only slightly in 70 days but when kept 43 days longer germinated very well, illustrating the effect of continued floating on water. Spores collected December 2, 1916, germinated somewhat in 3 days. From 5 to 40 per cent of the spores collected December 27, 1921, germinated in 6 to 11 days, but only a few spores of a collection made November 22, 1921, germinated in 14 days. Results of tests with spores collected January 5, 1918, were similar to those for December 27, 1921, but practically 100 per cent of spores collected February 26, 1918, germinated in 5 days while a very high percentage

of spores collected April 6, 1917 and 1921, would germinate in one day. Germination in the latter case began in less than an hour and some sporidia were produced within two hours.

Spores collected October 11, 1916, and kept in an herbarium till April 18, 1917, germinated somewhat in 38 days, and also spores collected December 2, 1916, and kept as herbarium material germinated in 2 days, March 7, 1917. This indicates that the date of collection in the fall or early winter may influence germination after keeping over winter in a warm room. But that neither a rest period nor low temperature is necessary for germination is indicated by a test of spores from a living plant in the greenhouse collected March 12, 1917, the spores of which germinated to some extent in from 6 to 8 days.

Teliospores of *Puccinia helianthi* would germinate feebly at 28° to 29° C. but the promycelia were abnormal and practically no sporidia were formed. Spores of *Puccinia windsoriae*, *Puccinia peridermiospora* and *Puccinia helianthi* would not germinate at 32° C. but when removed to room temperature gave positive results. Spores of *Puccinia helianthi* after floating on water at 38° C. for 48 hours failed to germinate, but withstood drying for 5 days at 38° C. and still germinated. Temperatures above the maximum delayed the time for germination to begin without inhibiting it completely; for example, spores collected April 6, 1917, and tested April 23, germinated well (++++) in one day at room temperature but failed to germinate in 52 hours at from 32° to 33° C. However, when this culture was removed to room temperature some germination (++) occurred in 24 hours.

Spores collected December 27, 1921, were tested in water, in m/15 KH_2PO_4 (pH 4.6) and in a mixture of m/15 KH_2PO_4 and K_2HPO_4 (pH 5.4). At the end of three days 1 ± per cent had germinated in water and 10 per cent in the solutions. At the end of 7 days the corresponding results were 5 to 10 per cent and 30 to 40 per cent.

Tests of spores collected April 6, 1922, were made April 10 to determine whether the concentration of KH_2PO_4 might influence germination, 1.25, 0.75, 0.50, 0.20, 0.10 and 0.05 per cent solutions and water being used. At the end of 3 hours inhibition was evident with the three most concentrated solutions. After 20 hours there was still marked inhibition in the strongest solution and possibly a little in the 0.75 per cent solution. An experiment was then performed April 11, with spores collected April 6, 1922, using first, 0.2 per cent KH_2PO_4, second a mixture of 0.2 per cent KH_2PO_4 and 0.05 per cent H_3PO_4, third 0.2 per cent KH_2PO_4 and 0.25 per cent K_2HPO_4 and fourth 0.2 per cent KH_2PO_4 and n/20

NaOH. The solutions had the hydrogen-ion concentration indicated below where the results are shown for 1, 2.5 and 19.5 hours.

pH	3.5	3.9	4.7	4.9	5.4	5.6	6±	6.5	7±	7.8	7.9	8.1	8.3	8.7±	Hr.
Germination	0	0	+	+	+	+	+	+	+	0	0	0	0	0	1
Germination	oc	oc	g	g	g	g	g	g	g—	g—	oc	oc	oc	vf	2.5
Germination	vf	f	vg	vg	vg	vg	vg	vg	g	g	f	vf	vf	oc	19.5
Sporidia	vf	vf	vg	vg	vg	vg	fr	fr	f	oc	oc	0	0	0	19.5

0= none; += germination; oc= occasional spore; g= good; vg= very good; f= few; fr= fewer; vf= very few.

The H-ion concentration of the solutions had not changed at the end of 20 hours excepting that the index for the last was 8.4. This experiment indicates that for *Puccinia helianthi* the limits of H-ion concentration for germination of teliospores are represented approximately by pH 3.5 and 8.4 and for production of sporidia by pH 3.5 and 7.8. Good or excellent germination occurred between concentrations represented by pH 4.6 and 7, while good sporidia production occurred within narrower limits (pH 4.6–6.5).

TABLE 8.

Germination of teliospores of Puccinia helianthi.

Collected	Tested	Incubation period	Result	Remarks
10/11/16	4/18/17	38 days	+ +	Kept in herbarium during winter.
12/2/16	3/7/17	2 "	+ +	Kept in herbarium during winetr.
"	12/9/16	3 "	+ +	
10/7/17	10/10/17	70 "	+	103 days, + + + +
10/16/17	11/10/17	39–56 "	+	
3/12/17	3/12/17	6–8 "	+	From living plant in greenhouse.
4/6/17	4/7/17	1 "	+ + + +	
"	4/23/17	1 "	+ + + +	1 hour, + +
"	4/28/17	2 "	+ + + +	Dried 5 days at 38° C.
5/5/17	5/7/17	1 "	+ +	Most spores had germinated out-doors.
10/22/18	10/22/18	4 "	+	
1/5/18	1/7/18	7 "	+ + + +	50+ per cent.
"	1/12/18	3 "	+ + + +	90 per cent; 3 days wet, 2 day dry, 1/7–1/12
2/26/18	2/27/18	1 "	+ +	2 days + + +; 5 days, 100 per cent.
4/11/18	4/12/18	19 hours	+ + + +	
11/22/21	11/22/21	14 days	+	
12/10/21	12/27/21	6 "	1 + per cent	In m/15 KH_2PO_4 (pH 4.6) 20+per cent.
12/27/21	"	6 "	5–10 "	In m/15 KH_2PO_4 (pH 4.6) 30–40 per cent.
"	"	11 "	40 "	In m/15 KH_2PO_4 (pH 4.6) 40 per cent.

DISCUSSION AND SUMMARY

These records for germination of teliospores of 10 species of rusts collected and tested at Columbia, Missouri, show that they all germinated in December or earlier as follows:

TABLE 9.

Germination of teliospores of rusts.

	Collected	Tested	Incubation period	Result	Remarks
Phragmidium potentillac-canadensis	10/7/17	11/3/17	5 days	+	Not tested earlier
Puccinia andropogonis	12/27/21	12/29/21	2 "	+	
Puccinia asparagi	12/27/21	12/27/21	6 "	+	Not tested earlier
Puccinia helianthi	10/ 7/17	10/11/17	70 "	+	
	10/22/18	10/22/18	4 "	+	
Puccinia menthae	11/22/21	11/22/21	15 "	+ + +	
Puccinia peridermiospora	12/17/18	12/17/18	35 "	+ + + + +	
Puccinia ruelliae	9/10/18	9/13/18	39 "	+ + +	
Puccinia sorghi	12/27/21	12/29/21	5 "	50 ± per cent	Not tested earlier
Puccinia sydowiana	12/27/21	12/27/21	22 "	+	
Puccinia windsoriae	12/17/18	12/17/18	35 "	+ + +	

In 4 cases tests were not made earlier than the dates given. It seems probable that in the case of 3 of these species (*Phragmidium potentillac-canadensis*, *Puccinia asparagi* and *Puccinia sorghi*) germination would have occurred considerably earlier.

As the season advances there is a decided increase in the percentage of spores that will germinate in a given time. For example, teliospores of *Puccinia helianthi* required 103 days for a high percentage of germination in October, 11 days in December, 7 days in January, 5 days in February and less than one day in April. Some of the other species (*Puccinia menthae*, *Puccinia peridermiospora*, *Puccinia windsoriae*) behaved similarly. For the remaining forms the data were not sufficient to determine this point.

The time required for germination to begin decreases as spring approaches. This point is well illustrated by the behavior of *Puccinia helianthi*. Spores of this rust germinated slightly in 70 days October 10, 1917, but better in 6 days December 27, 1921, in one day February 27, 1918, and in one or two hours in April 1917 and 1922. The data presented indicate that the same is true for *Puccinia peridermiospora*, *P. windsoriae*, *P. menthae* and *P. ruelliae*. A small percentage of spores of *P. perideriospora* germinated in 32 days January 7, 1918, in 15 days March 10, 1920, and in 2 days April 7, 1917. Spores of *P. windsoriae* failed to germinate in 36 days September 13 and in 61 days October 16, 1918, but a small percentage germinated in 41 days December 29, 1921, in 35 days December 17, 1918, in 39 days January 7, 1918, and in 2 days

April 30, 1918. From 1 to 5 per cent of spores of *P. menthae* germinated in 15 days November 22, 1921, but 20 to 30 per cent germinated in 2 days December 29, 1921.

After germination in a culture has begun it will generally continue for a considerable period of time. This may be seen in the following table.

TABLE 10

Effect of length of incubation period on percentage of germination of teliospores.

	Tested	Incubation period	Result	Incubation period	Result
Phragmidium potentillae-canadensis	11/3/17	5 days	+	28 days	100—per cent
Puccinia asparagi	12/27/21	6 "	+	17 "	5± per cent
Puccinia helianthi	2/27/18	1 "	+	5 "	100—per cent
Puccinia menthae	12/29/21	2 "	20–30 per cent		
	12/27/21			6 "	80 per cent
Puccinia peridermiospora	2/7/22	13 "	10 per cent	20 "	100—per cent
Puccinia ruelliae	9/13/18	39 "	+ + +	53 "	+ + + +
Puccinia sorghi	12/29/21	2 "	5 per cent	11 "	90± per cent
Puccinia sydowiana	"	22 "	Rare	41 "	2–5 per cent
Puccinia windsoriae	"	10 "	+	41 "	5± per cent

These results demonstrate the effect on germination of continuous wetting (floating on water) of the spores. On examining the tables on germination of *Puccinia ruelliae*, *P. peridermiospora*, *P. windsoriae* and *P. andropogonis* it will be seen that there is little difference between the effect on germination of continuous floating on water and alternate wetting (soaking) and drying. If the period of wetting and drying be added to the incubation period in most cases it is approximately the same as that required for germination when spores are simply floated on water. In other words the effect on germination of the two treatments is essentially the same. The main factors concerned in these treatments are unknown. While the effect of alternate wetting and drying has been known for some time, the writer has found no records in the literature concerning the effect on germination of continuous floating on water.

Teliospores evidently may also change under dry conditions at room temperature. For example only 1 to 5 per cent of spores of *Puccinia peridermiospora* collected March 9, 1920, and tested the next day, germinated in fifteen days. However spores of the same material germinated very well (++++) in 6 days after being kept dry at room temperature until April 8. Moreover spores of *Puccinia andropogonis* collected and

tested as above failed to germinate in 23 days in the first test, but considerable germination occurred in 2 to 4 days in the second one.

As compared with germination in distilled water that in solutions with higher H-ion concentration (pH 4.6 and 5.4) was retarded in the case of *Puccinia asparagi*, *P. sorghi*, *P. ruelliae* and *P. menthae*. On the other hand it was improved for *P. helianthi*. *Puccinia helianthi* will germinate in solutions having a wide range of H ion concentration (approximately pH 3.5–8.4) but the limits for good sporidia production are narrower (approximately pH 4.6–6.5).

CONCLUSIONS

Teliospores of certain rusts having a more or less definite rest period may germinate to some exfent in December or earlier. Germination is especially favored by prolonged floating on water and by alternate wetting (immersion in water) and drying. As the season advances there is a marked increase in the percentage of spores that will germinate and a decrease in the time necessary for germination to begin, and for complete germination (highest possible percentage of germination).

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BITTER PIT IN APPLES: THE CRUSHED CELL THEORY.

D. A. HERBERT

McAlpine's five voluminous reports[1] on his investigation of the bitter pit question present a tremendous amount of detailed research into the climatic and other factors operating in the production of the disease and also put forward methods for its control. The actual explanation of the pitting, however, is not convincing, and the Bursting Cell Theory is unsound. In the following account, the crushed Cell Theory is put forward a being the explanation of the trouble. It does not involve the assumption that prior to death the pit cells must swell to the bursting point.

A brief outline of the characteristics of the disease is necessary to stress the main points on which the interpretation of the cause rests.

The presence of sunken dark spots on the surface, resembling hailmarks except that they are scattered over the entire surface instead of only on one side is the first evidence of bitter pit. In the case of the Rome Beauty the diseased areas occur in patches, which sometimes converge around the apple. In this condition the disease is known as confluent pit or crinkle. As McAlpine points out, the pit originates in the vascular network just beneath the skin, the blemishes appearing to a lesser extent deep-seated in the flesh of the apple. In section it is seen that the pits consist of collapsed dead cells *filled with starch*.

The Bursting Cell Theory as summarized by McAlpine[2] is as follows:

When there is an extra rush of sap following on dry conditions, the rapidly swelling pulp cells at the external boundary burst the vascular network at localized spots and the sap pressure which is sufficient to rupture the enveloping network also bursts the thin-walled pulp cells at these particular spots and death of the cells ensues. Briefly, it may be stated that rapid alternations between dry and moist conditions, combined with fluctuating temperatures, during the growing stages of the fruit are the enciting causes of bitter pit.

There are several points in this theory which are not supported by the following facts:

[1] McAlpine, D. Bitter pit investigation. First Progress Rept. 1911–12. 197 p., 33 pl. Literature, p. 111–117. Fifth Progress Rept. 1915–16. 144 p., 37 pl. Literature, p. 67.

[2] McAlpine, D. Bitter pit in apples and pears: latest results in preventive measures. Phytopath. **11**: 366–370. 1921.

a. Sunken spots constitute the first external symptoms.

b. The skin is intact over the pit areas.

c. The vascular bundles frequently extend through a pit spot and supply normal tissue beyond.

d. Starch is present in the pit cells.

e. The tensile strength of cellulose is sufficient to eliminate the possibil ty of bursting.

These points will be dealt with below but in view of their interdependence they will not be discussed under separate headings.

It is generally accepted that the fluctuation in water supply is the factor primarily responsible for bitter pit. The point in question, however, is in what manner the diseased patches are produced. The rush of sap to the apple after a fall of rain following a dry spell causes a distension of the parenchymatous pulp cells. If the cells in any particular pit area were to distend to such an extent as to cause their bursting the result should be the local production of wart-like bodies before the bursting and subsequent death took place. As a matter of fact, a sinking of the tissue at the affected points constitutes the first external symptoms observed. In the case of Dunn's Seedling (Monroe's Favourite) the flow of sap after a rain following a dry season is sufficient to cause the expected distension of the pulp cells to take place. The force of the distension is sufficient to cause a bursting of the skin. No bursting of the cells takes place within the apple; in fact, it is difficult to see how this could be possible because the expansion of adjacent cells would bring the tissue into a state of static equilibrium and the only relief possible for the increased pressure would be either a bursting of the skin (as in the Dunn's seedling) or a crushing of some of the cells. There is not sufficient internal space in the apple for the expansion of cells to the bursting point. If cells are dissected out and placed in water the amount of water imbibed is not sufficient to cause bursting although under this condition they are freed from the natural obstacle of adjacent turgid cells which is found in the unruptured apple, and which helps to prevent their expansion. An osmotic pressure of 100 atmospheres when entirely borne by the cell may cause bursting but this is enormously beyond anything met with in the cells of the apple.[1] In this case there is the further factor of the pressure from neighboring cells so that bursting of these internal cells is impossible.

The vascular tissue may frequently be traced through a pit and found to be supplying healthy tissue beyond. This would not be the

[1]Dixon, H. H. Transpiration and the ascent of sap. Progressus Rei Botanicae **3**: 1–66. 7 fig. 1909.

case if the vascular net-work were burst as the Bursting Cell Theory assumes.

The presence of large quantities of starch in the dead cells is another point to be considered. Pitting takes place in the apple for the most part at the time when the starch is being converted into sugar. It is evident that when there is a sudden rush of sap into the apple, the cells which have had their starch converted into sugar will swell to a greater extent and more rapidly than those cells wh ch are still supplied with starch. The rapid distension of the cells s resisted on the outside by the skin. Their force of expansion results in the crushing of those cells whose starch transformation is backward. This accounts for the presence of quantities of starch in the dead pi cells.

It is suggested, though it remains to be proved, that immunity from bitter pit, which is shown in such varieties as Yates, may be due to the uniform transformation of the starch to sugar through the tissue. If such is the case there would be no small clusters of cells far enough behind in their starch transformation to be crushed by neighbouring cells of a higher osmotic pressure.

SUMMARY

1. The Bursting Cell Theory does not satisfactorily explain the ultimate cause of bitter pit in apples.

2. The theory is advanced that the affected cells have been killed by being crushed by neighbou ing cells having higher osmotic pressure due to their higher proportion of sugar.

3. The presence of starch in quantity in the pit cells, the intact skin over the pitted areas, the first external symptoms being a sinking of the skin, and the fact that a vascular bundle may run through a pit area and supply healthy tissue beyond supports this view.

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STAINING GERMINATING SPORES

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Investigators have experienced difficulty in making permanent slides showing well differentiated protoplasmic contents of spores, sporidia, promycelia and germ tubes from germinating spores. Lutman (3, p. 1208) transferred germinated spores to microscopic slides bearing albumen fixative, treated the culture with Flemming's weak killing solution and proceeded with the usual staining processes. Osner (4, p. 203) treated his materials similarly but germinated the spores on the albumen fixative. The writer has employed the above methods together with those described by Harper (1) and other workers. The spores either failed to germinate, were lost in the staining and washing processes or lost their turgidity and plasmolyzed so much that the nuclear contents of the germinating parts could not be definitely distinguished. None of the above methods seemed to give assurance that the cultures could be securely retained on the slides for mounting in balsam. This was found especially difficult when iron-alum-haematoxylin stain was used on account of the number and length of washings. Kniep (2, p. 294) employed a method similar to the one herein described and it overcomes many of the difficulties experienced in the manipulations of the other methods. The procedure used by the writer is as follows:

1. Germinate the spores in distilled water, sterilized water or other desirable solutions placed on a slide within a petri dish lined with damp filter paper. Sterilize all apparatus before using and centrifuge the spores in the ordinary way to reduce contaminations.

2. When the proper stage of spore germination has been reached, place five drops of Flemming's weak killing solution on the culture, return it to the petri dish and allow to stand 15 to 30 minutes.

3. Draw the killing solution from the culture by rotating the edge of a circular piece of filter paper held in the margin of the liquid. Wash the culture three times by adding each time several drops of distilled water to the surface and blotting as before. The best results were obtained by allowing the culture to stand for one hour in a fourth washing solution.

4. After blotting off as much water as possible, allow the remaining water to evaporate until the culture has just reached the moist stage. Submerge the slide in water-free ether in a Petri dish and rotate it until all the water is absorbed.

5. Remove the slide from the ether, hold it in a slanting position and pour a two per cent solution of parlodion (or any other good grade of celloidin dissolved in equal parts of absolute alcohol and ether) over the culture beginning with the upper and finishing at the lower end. It is best to pour just enough of the parlodion solution to cover the culture surface of the whole slide and not drain any from the slide.

6. Allow the parlodion to air-dry in a horizontal position until the mottling has nearly disappeared. No difficulty was experienced in proceeding with the next step without drying the paroldion, but when allowed to dry too long, the parlodion film had a tendency to part from the slide. With India ink, label the culture on the parlodion film. However, while the water is evaporating from the culture, the labeling may be placed on one end of the glass slide and, together with the culture, floated with the parlodion.

7. With a pipette, flood, the parlodion film with five changes of alcohol in the following order: 96, 85, 70, 50, 35 per cents and transfer the slide to the stain. The writer secured the best results with the iron-alum-haematoxylin stain. Submerge the slide in a 4 per cent iron-alum mordant.

8. After the culture remains in the mordant for one hour, wash it 5 minutes in running water and then transfer to a 0.5 per cent aqueous solution of Haidenhain's haematoxylin for 2 hours or more.

9. Wash the slide 10 minutes, destain with a solution composed of equal parts of the mordant and water until the parlodin still retains some of the haematoxylin stain. Examination under the microscope will reveal the proper differentiation which was generally found to be at this point.

10. After washing the slide 5 hours, flood the culture with five changes of alcohol in the following order: 35, 50, 70, 85, 96 per cents. Two treatments were employed in the 96 per cent alcohol and the slide well drained. Absolute alcohol should not be used because it dissolves the parlodion and frees the spores.

11. Flood the culture with Eycleshymer's clearing solution composed of equal parts of oil of bergamot, cedar oil and a solution derived from the deliquesced crystals of carbolic acid. Pour off the clearing solution at once and cover with fresh clearing solution which is allowed to remain until the parlodion film is perfectly clear or about thirty minutes.

12. Drain off the clearing solution and mount in balsam.

If the parlodion film parts from the glass slide at the end of any process, the film may be straightened out and floated on the slide under water. If the slide is then drained and ether dropped on the ends of

the parlodion film and more parlodion solution poured over the ends, the culture will remain secure. With careful manipulation every culture can be successfully mounted.

Permanent slides showing well stained germinating spores of the following genera have been secured by this method: Aspergillus, Ascochyta, Doassansia, Gymnoconia, Gymnosporangium, Penicillium, Phragmidium, Puccinia, Sordaria, Tilletia, Uromyces, Urocystis, Ustilago and Venturia. The iron-alum-haematoxylin stain gave better results than the triple stain. However, the hyaline germ tube walls of the smuts failed to take the haematoxylin stain.

If mounted slides are not stained sufficiently or fade, the balsam may be removed with xylol, the culture treated with the alcohols and restained as previously described. The spore membrane may be made more translucent by treating the culture from 6 to 12 hours in a 2 per cent solution of hydrogen peroxide. The positive assurance that the culture may be retained and the ability to properly stain the materials are the principal merits of this method.

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(4) Osner George A. Leaf smut of timothy. Cornell Univ. Agr. Exp. Sta. 381: 189–230. 1916 fig. 45–58, pl. XVII. Bibliography p. 226–230.

NOTE CONCERNING THE DECAY OF WESTERN YELLOW PINE SLASH CAUSED BY POLYPORUS VOLVATUS PECK

HENRY SCHMITZ

WITH ONE FIGURE IN THE TEXT

The question of brush disposal in forest operations both in relation to the fire menace and in relation to forest sanitation is one of the most important questions of general forest management in the Inland Empire. In a comprehensive study of the menace involved in neglect of burning slashings after logging operations, Hubert (2) has found that all of the most destructive wood rotting fungi operating in the forests of the north-

west develop fruiting bodies on their hosts after the tree is cut. We are indebted to Weir,[1] working alone and in collaboration with Hubert, for much of the fundamental data dealing with the pathological aspects of forest management in the northwest. Meinecke (3) and Boyce (1) have also made important contributions to our knowledge on this subject.

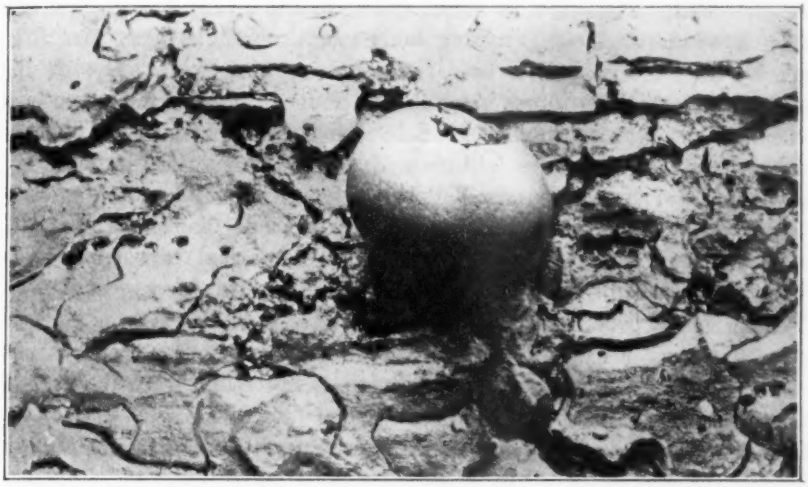

FIG. 1. Fruiting Body of *Polyporus Volvatus* on a Burnt Yellow Pine Slashing.

In going over the literature, the writer has found only a very few references made to *Polyporus volvatus* Peck in relation to slash or wood decay. Meinecke (4) however, says in part: "*P. volvatus* causes a slow working, rather superficial gray rot, is not considered parasitic; it does not endanger living trees, but is very common." Both Zeller[2] and Schmitz (5) have suggested the possible parasitic nature of this fungus. The present note reports briefly the general occurrence of *Polyporus volvatus* particularly on western yellow pine slash.

During the past several years, the writer has noted fruiting bodies of this fungus on cut western yellow pine logs, saplings and poles, but little attention was paid to the observation. This spring, however, an area several acres in extent has been under observation. This area had a young stand of western yellow pine in the small pole stage. The area

[1] See Hubert, E. E. *loc. cit.* pages 55–56 for a list of these papers.

[2] Unpublished data.

was slashed in the late summer and winter of 1920 and broadcast burned in June, 1921. Although the tops were entirely burned, the bark on the trunks of the trees was only slightly charred. By the spring of 1922, fruiting bodies of *P. volvatus* were common on not only the trunks of trees which had their bark charred but also on the trunks of trees missed by the fire. It is possible that the bark of trees in the neighborhood of the fire, although showing no visible injury may have been devitalized by the heat. Infection in these trees suggests that *P. volvatus* is at least a weak parasite. Fruiting bodies of the fungus were also found nearby on western yellow pine fence posts which were set with their bark still on. These posts are not sufficiently close to the burned area to have been in any way affected by the heat of the fire. Hubert (2) showed that logs in close contact with the ground develop sporophores more readily than logs raised above the ground. Since many of the tree trunks which were considered in the present discussion are not in close contact with the ground, it is problematical how many years sporophores will be produced. Text figure 1 shows the appearance of a fruiting body protruding through the charred bark.

A microscopic examination of the wood revealed the fact that it is in the incipient stages of decay. Fungous mycelium extends generally throughout the wood. Of course, it is a question if this mycelium is that of *Polyporus volvatus*, but since the fruiting bodies of no other fungi were visible, it is not at all unreasonable to believe that it is *P. volvatus*. Experiments are now in progress in this laboratory dealing with the wood destroying proclivities of this fungus.

In conclusion, the writer ventures the opinion that *Polyporus volvatus* may be a much more important fungus than many forest pathologists now suppose.

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PHYTOPATHOLOGICAL NOTES

A Fungus destructive to asphalt shingles.—Views (p. 498) are presented herewith of the two sides of an asphalt shingle heavily overgrown and penetrated by a fungous mycelium. When submitted to the writer some two years ago the statement was made that the reshingling of a roof had been necessitated by the destructive action of this fungus. Later reports indicate that stock in storage is sometimes similarly damaged. The asphalt shingles in question are based on a "felt-paper" similar to that used under carpets. The felt is pulled through liquid asphalt at high temperature and thus saturated and after the excess asphalt is pressed out the shingle is slightly air-cooled and given a coat of natural asphalt, and slate applied on one side. The other side is finished with a mixture of talc and mica for the purpose of destroying the adhesive character of that side of the shingle. The fungus in question is a basidiomycete with mycelium of the general character of that of *Merulius lachrymans*. It grows vigorously, not only over the surface, but it actually penetrates the shingle, subsisting presumably upon the felt paper on which the shingle is based.—F. L. Stevens.

The bacterial pathogen of corn stalk rot.—In view of the rather widespread interest in the bacterial stalk rot of corn, first described from Arkansas (Phytopath. **11**: 74–79, 1921), and now reported from about 8 different states, it seems desirable to present a preliminary description of the pathogen. As will be shown in another publication, the organism has not been previously described and for convenience will be briefly characterized at this time.

Pseudomonas dissolvens n. sp.[1]

Short, plump, rapidly growing rods, motile by means of a single polar flagellum, bluntly rounded at both ends, occurring usually singly or in pairs, occassionally in short chains, average measurements 0.7–1.2 × 0.5–0.9μ, capsules present, no irregular forms or spores observed, white on most solid media; colonies on nutrient agar poured plates, testing pH 7.0, round, margins entire, white, opaque, glistening, consistency of melted butter, emitting a strong order of decaying vegetable matter; gelatin not liquified; acid and gas produced on most nutrient media; diastatic action noticeable; indol and ammonia are produced; Loeffler's blood serum not liquified; nitrates are reduced; milk clears at the end of the fourth day, coagulation delayed but marked on the sixth

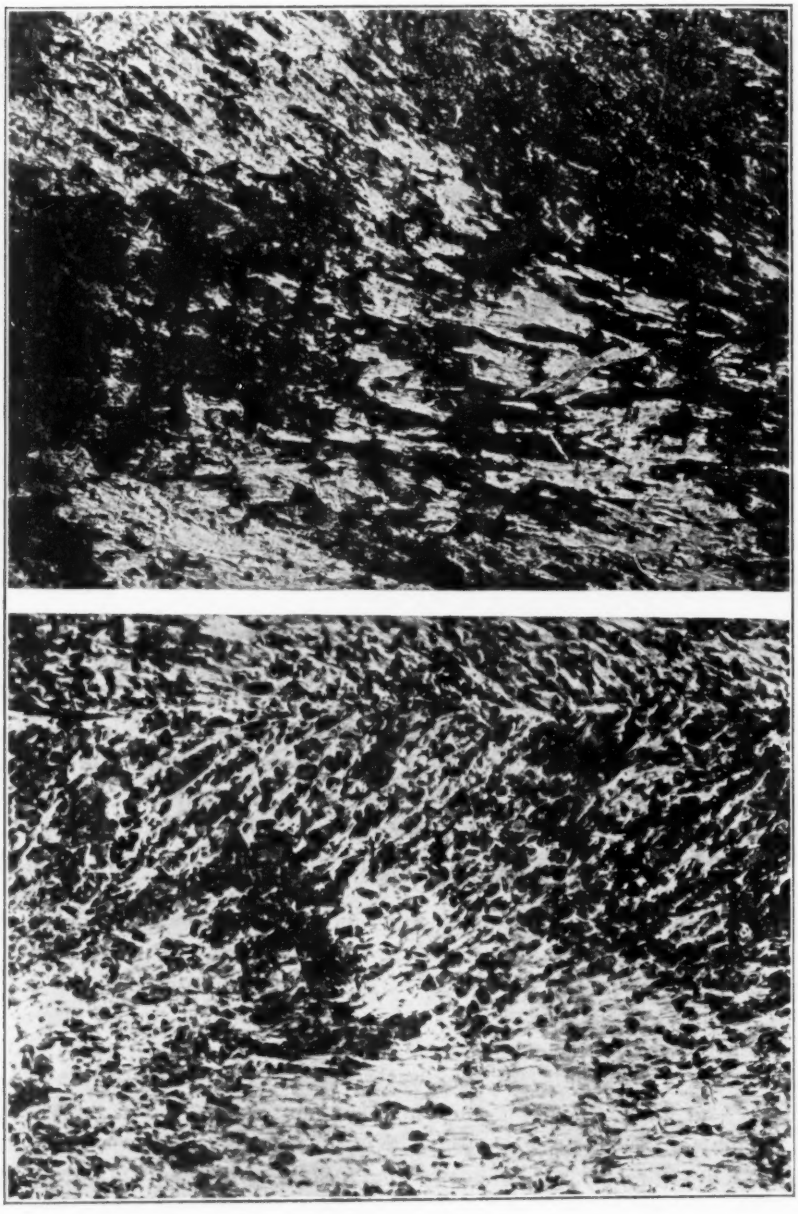

FIG. 1. Photographs showing asphalt shingles penetrated by fungi, viewed from above and below.

day; no growth in Fermi's solution; growth in Uschinsky's solution good. The index number, using the most recent chart endorsed by the Society of American Bacteriologists (1920) is 5322-32220-1111.

Pathogenic on *Zea mays*.—H. R. ROSEN.

Whetzel resigns headship—deplores present system of administrative tenure.—Professor H. H. Whetzel who has been for fifteen years head of the Department of Plant Pathology of Cornell University College of Agriculture retired on July 1 from the administrative leadership in order to devote his time and energies more fully to teaching and research together with the immediate preparation of one or more text books. Dr. L. M. Massey who has been acting head for the past year during Professor Whetzel's absence in Bermuda succeeds to the permanent position.

Cornell was the first American university to establish an independent Department of Plant Pathology and has continued as the largest development in its field. When I last visited Cornell a year ago the Department had, in addition to what were doubtless the largest undergraduate classes of any like department in America, an enrollment of twenty-six graduate students. It has during the last thirteen years granted twenty-four Ph. D. degrees with major interest in plant pathology. These men are now scattered in fifteen institutions in the United States. This of itself constitutes a record of service in proportion to opportunity which I can liken, within the range of my personal acquaintance, only with that of the man under whom the undergraduate work of Whetzel and many of the Cornell phytopathologists and botanists was taken—the late Professor M. B. Thomas, of Wabash College. Whetzel has, in all his work, shown a genius for meeting old problems in a new way in the field of phytopathology.

In a letter to his dean setting forth his reasons for wishing to retire from the headship, Whetzel says:

"I am convinced that with rare exceptions no man should be allowed to head a department for more than fifteen years. He should not serve less than ten, unless he is a failure—then the sooner he steps out or is removed, the better. The head of a department who cannot in ten or fifteen years put over a big administrative project deserves no further time or opportunity.

"When a young man I was ambitious, like most of my colleagues, for administrative opportunity and position. Increased salary was, of course, a strong factor, but by no means the strongest. I like the

[1]The name of the organism, using the classification proposed by Smith (Bacteria in Relation to Plant Diseases 1: 171, 1905) is *Bacterium dissolvens* n. sp.

game. I played hard at it. I feel I was in a reasonable measure successful, but my chief interest has always been teaching and research, particularly teaching. Few men can do both administration and teaching, let alone research also with full justice to all. After ten years of administrative work I long to give my time and energies fully to teaching and research work. As you know, I have for four or five years urged you to relieve me of the headship that I might devote myself wholly to my students and my investigations. I feel that another ten years would not only measure the span of my administrative usefulness but would also end any possibility of a successful return to teaching or research.

"One of the most disastrous features of long tenure headship is its effect on ambitious and able young men in the staff. Such men see little hope of administrative opportunity within a reasonable period of time and either leave the institution for a better position elsewhere or become discouraged, discontented, often intriguing and disloyal to the chief. The result in the latter case is sure to be disastrous to some one, usually to the young man himself, often to the department and to the institution, not infrequently to the good name and prestige of the head of the department himself.

"Every university man deserves to opportunity to try for administrative position early in his professional career. The advancement of a man to the headship after he is forty is, in my opinion, rarely warranted. Youth has generally borne the banner of progress in university development as well as in other fields. 'Young men for action, old men for council' and the university may have both, but not to the fullest advantage under our present system of administrative tenure.

"I have had the direction of this department in the College from its beginning. I have seen it expand in fifteen years into one of the largest in the institution. I love it as a father loves his child. I want to see it grow and prosper. I could not bear to see it stagnate or die, as not infrequently happens when one retains administrative responsibility too long. I am sure a younger man can carry it forward farther and with greater success than I could now. Successful administration demands long hours, large personal sacrifices, great energy, and the resiliency of youth. I have given as generously and as completely of these as I could for a decade and a half. I feel that I have earned the privilege of now devoting myself to those phases of my profession nearest my heart. I know that I can teach, I wish to do it. I think that I can do research work, and I wish to try it."—L. R. JONES.

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